The University of Alberta
Printing Department
Edmonton, Alberta

THE UNIVERSITY OF ALBERTA

COPPER SUPPLEMENTATION OF SWINE DIETS AS INFLUENCED BY PROTEIN SOURCE AND TRACE MINERAL SUPPLEMENTS

by

© TITUS ADEBOWALE OMOLE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

ANIMAL NUTRITION

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL, 1973

ABSTRACT

Two experiments were conducted to study the effects of supplementing swine diets with 0, 125 or 200 ppm copper to either 40 kg liveweight or a market weight of 77 kg.

In Experiment 1, 76 pigs, 38 each of gilts and barrows, were fed isocaloric, isonitrogenous diets with protein supplements of either soybean meal (SBM), low glucosinolate rapeseed meal (RSM), from Brassica napus cultivar Bronowski rapeseed, or a combination of SBM and RSM. Each dietary treatment was further supplemented with either 0, 125 or 200 ppm copper. Supplemental iron and manganese, with or without copper supplements, were also included in some dietary treatments. In Experiment 2, 48 pigs equalized as to sex, were fed protein and copper supplements similar to those fed in Experiment 1, except that RSM from B. campestris seed was used. Some of the diets supplemented with 200 ppm copper were further supplemented with 200 ppm zinc.

In Experiment 1, feed intake and efficiency of feed utilization were not significantly influenced by levels of copper or by source of protein. Rate of gain averaged 0.66 kg per day for pigs fed 200 ppm copper throughout which was superior, though not significantly, to that of any other treatment, suggesting an advantage to using 200 ppm dietary copper compared with 0 or 125 ppm copper and of keeping the 200 ppm copper in the diet to market weight.

Supplemental iron and manganese had no significant influence on results. Source of protein had no influence on rate of gain suggesting that a low glucosinolate RSM could replace SBM on an isonitrogenous and isocaloric basis. In Experiment 2, feed intake, rate of gain and efficiency of feed utilization were not significantly affected by supplemental copper or copper and zinc, protein source or sex.

Digestion coefficients were not significantly affected by copper supplements, protein source or sex in the two experiments.

In Experiment 1, pigs receiving dietary copper had lower backfat thickness than pigs receiving 0 level supplemental copper. Dressing percentage and loin area were slightly increased by dietary copper in the two experiments. Carcass characteristics were not significantly influenced by source of protein or sex.

Copper concentrations in the liver and kidney increased with increase in dietary copper levels. Reductions in liver and kidney copper concentrations were brought about by withdrawing copper supplementation at 40 kg liveweight or by supplemental zinc. Dietary iron and manganese did not influence the liver and kidney copper stores. Muscle and fat copper levels were not influenced by dietary copper, iron and manganese or zinc.

The proportions of unsaturated fatty acids were higher in the depot fat of pigs receiving copper supplementation or RSM compared with those receiving no copper supplement or those fed SBM respectively.

ACKNOWLEDGEMENTS

I wish to thank Dr. L.W. McElroy, Chairman of the Department of Animal Science, for placing the facilities of the department at my disposal. I am particularly indebted to Dr. J.P. Bowland, Professor of Animal Nutrition, for his helpful criticisms and suggestions during the course of this study and especially his assistance in the preparation of this manuscript. The assistance of Dr. R.T. Hardin, Associate Professor of Poultry Genetics, and of Mr. R. Weingardt in the statistical analyses of the data is gratefully acknowledged.

The assistance and willing cooperation of Mr. Graeme Stephens, Herdsman at The University of Alberta Swine Research Station and his staff are greatly appreciated. The assistance of Miss Mira Zastavnikovich in determination of fatty acids is acknowledged.

Personal encouragement from Professor A.A. Adegbola, Dean of Agriculture and the Deputy Vice Chancellor, University of Ife is hereby acknowledged.

Financial support from the National Research Council of Canada in the form of a Grant-in-Aid to my supervisor and a supplementary allowance from the University of Ife, Nigeria is hereby acknowledged. The rapeseed meals used in these studies were provided by The Rapeseed Association of Canada.

TABLE OF CONTENTS

Page

Page

Page

LIST OF TABLES

Page

Page

LIST OF FIGURES

INTRODUCTION

It has been well established that copper is essential for the normal growth, development and functioning of plants and animals. Diets composed of normal feedstuffs seldom contain less than 5 ppm of copper, a level considered adequate in terms of copper requirements of the pig (NAS-NRC, 1968). Beyond the suckling stage therefore, there is little likelihood of true copper deficiency being encountered. Indeed, supplementation of most livestock and human diets is not considered necessary and if practiced may result in toxicity.

The pig can tolerate and often respond to high level copper supplementation of the diet. The response, when it occurs, is manifested by improved average daily gain and feed conversion (Braude, 1965). Copper supplementation is usually accompanied by high concentrations of copper in liver and kidney. Contradictory reports exist in the literature regarding the related effects of copper, zinc, iron and manganese on performance and copper stores of pigs. Increasing the dietary level of zinc above that found in normal diets has been shown to decrease the amount of copper stored in the livers of pigs (Allen et al., 1958; O'Hara, Newman

and Jackson, 1960; Hanrahan and O'Grady, 1968). However, other workers have not been able to reduce the copper stores of rats and pigs by increasing dietary zinc (Kulwich et al., 1953; Cox and Hale, 1962; Kinnamon, 1966; Gipp, Pond and Smith, 1967). Also high dietary levels of iron have been shown to reduce liver copper stores in pigs fed excess copper (Kainski et al., 1967). There is no single explanation for the varied responses of pigs to the addition of supplemental zinc and iron to copper-supplemented diets.

From the results of more than a decade of active research on the value of rapeseed meal (RSM) as a protein supplement in swine rations, a maximum level of 5% RSM of the types now available commercially has been recommended to be fed to starting, growing and finishing pigs (Bowland et al., 1966). Higher levels are not recommended because rapeseed contains an enzyme, myrosinase, which can hydrolyze the glucosinolates present in the seed to compounds potentially harmful to animals. In view of the fact that new varieties of rapeseed, low in glucosinolate levels, will be commercially available in Canada within the next few years, studies to compare the relative values of low glucosinolate RSM and standard RSM with soybean meal (SBM) are desirable.

The present studies were undertaken between March, 1971, and January, 1973, to obtain further information on the effects of copper supplementation on the growth performance, carcass characteristics and the depot fat composition of pigs; to examine

the related effects of copper, zinc, iron and manganese on performance and copper stores of pigs and to evaluate two types of RSM as partial or full replacements for SBM in the diets of market pigs.

LITERATURE REVIEW

BACKGROUND INFORMATION

The first report that supplementation of diets with copper sulfate improved growth rate in pigs was that of Evvard, Nelson and Sewell (1928), but very little attention was given to this report until Braude (1945) described how pigs chewed copper rings which were a part of the structure of their piggery. To test whether this apparent craving for copper reflected a dietary deficiency, 8 and 14 week-old pigs were fed a basal diet containing about 5 ppm of copper and some were given a daily supplement of 50 mg copper in the form of copper sulfate solution, poured on their rations. During the first eight weeks of one experiment, the addition of copper improved both rate of gain and efficiency of food conversion by about 8%, but data from the second experiment did not confirm this. Braude (1948) later reported that weaned pigs given a choice of mineral licks preferred those containing copper. In another trial, two pigs ate greater amounts of a meal mixture when it contained copper sulfate to supply 675 ppm copper.

Mitchell (1953) offered six litters, consisting of 55 pigs, free choice access to two creep-feed type diets. The basal diet

contained 10 ppm copper while the second was supplemented with copper (as $CuSO_4.5H_2O$) to a level of 150 ppm. Feed consumption of the two diets was measured from two weeks of age until weaning at eight weeks of age. During the six week period the pigs consumed 54.4 kg of the basal diet and 161.0 kg of the copper supplemented diet. He concluded that the diet containing 150 ppm Cu was more palatable. A later experiment involving 300 pigs and the same level (150 ppm) of dietary copper supplementation failed to confirm this observation (Barber et al., 1955a).

EFFECTS OF COPPER SUPPLEMENTATION ON LIVE PERFORMANCE

A suggested growth promoting property of copper was presented by Barber et al. (1955b) who studied the effects of a commercial mineral mixture XF containing 4% copper sulfate, added at a dietary level of 2.5% to supply 250 ppm Cu, to a ration fed to scale to weanling pigs for a period of eight weeks. While there were no statistical differences in weight gains; feed consumption and efficiency of feed utilization were improved for those pigs receiving the supplement. In order to substantiate these findings, Bowler et al. (1955) carried out a field trial in which eight centers cooperated. At four of the centers two replicates of the test were run. The overall result of the twelve replicates showed a statistically significant improvement in the growth rate and a non-significant benefit in feed conversion efficiency when the mineral mixture containing copper was included in the diet. Mineral

supplement studies of swine rations at The University of Alberta in 1954 (Bowland, 1954) involving supplemental copper, manganese, cobalt, iron, magnesium and zinc fed individually or as a mineral mixture did not indicate any significant benefit in rate of gain or efficiency of feed utilization of the experimental animals receiving the supplemental minerals.

Lucas and Calder (1957) reported improved rate of gain and efficiency of feed conversion during the growing period from 20.0 to 47.3 kg in pigs fed diets supplemented with copper at a level of 250 ppm; however, between 45.5 and 91.0 kg no significant differences in these two parameters were found. Lucas et al. (1961) observed that the overall effect of copper on rate of gain and efficiency of feed conversion to market weight is a reflection of its effect during the growing period. Barber et al. (1957) reported that copper supplementation of diets fed to fattening pigs resulted in a non-significant increase in feed consumption but a significant increase in daily rate of gain.

This response in daily gain has been confirmed in many subsequent experiments (Hawbaker et al., 1961; Fagan et al., 1961; Bellis, 1961). In a large-scale coordinated trial, Braude et al (1962) recorded an improvement of 9.7% in growth rate and 7.9% in the efficiency of feed conversion when 0.1% copper sulfate was added to diets. Lucas (1964) noted that when pigs were fed to a scale based on liveweight, as is done in Europe, the major response to copper supplementation was prior to 45.5 kg liveweight;

however, if pigs were fed ad libitum, as is the practice in North America, responses also occurred between 45.5 and 91 kg liveweight. The latter response was related to increased feed intake. A series of studies conducted at The University of Alberta (Bowland, 1963) indicated that growing pigs responded more dramatically to copper or antibiotic supplementation of the diets prior to 34 kg liveweight than between 34 kg and 90 kg liveweight.

More recent observations (Castell and Bowland, 1968a; Hanrahan and O'Grady, 1968; Kline et al., 1971, 1972; DeGoey et al., 1971) agree with the earlier reports. Drouliscos et al. (1970) reported an improvement of 12% in growth rate of pigs fed fishmeal diets supplemented with 250 ppm copper. Braude (1965, 1967) has published extensive reviews of literature on the effects of copper on average daily gain and efficiency of feed conversion. All trials reported in the literature up to mid-1965, in which the performance of growing pigs receiving supplemental copper at a level of 250 ppm was directly compared with that of similar control animals, were summarized. This involved 83 trials and a total of 1215 pigs per treatment. Copper supplementation resulted in an overall improvement of 8.1% for growth rate and an overall improvement of 5.4% for feed conversion. A similar compilation of experiments involving copper supplementation in the U.S.A. was made by Wallace (1967). From his calculation, he observed that baby pigs or early weaned pigs respond to copper feeding in a

dramatic way. The average percent response in daily gain was 22.1% and in feed conversion 8.3%. In only three of the 43 comparisons did copper fail to improve weight gain. In only five of the 43 comparisons did copper fail to improve feed conversion. Growing pigs responded +6.5% in gain and +2.3% in feed conversion, while growing-finishing pigs responded +3.6% in weight gain and +1.1% in feed conversion.

Failure to obtain response to copper supplementation has been documented in more recent studies by Pond and Smith (1967), Bekaert et al. (1967) and Livingstone and Livingston (1968).

EFFECTS OF COPPER SUPPLEMENTATION ON CARCASS CHARACTERISTICS

In his review of literature, Braude (1965) concluded that the majority of reports concerned with copper supplementation of swine diets had indicated no adverse effects on carcass quality; however, a few reports had classified the occurrence of soft carcass fat attributable to dietary copper supplementation as a less favorable quality.

The observed effects of dietary copper supplementation on dressing percentage, backfat thickness and carcass length have been extremely variable. Allen et al. (1958, 1961) and Barber et al. (1961b) reported a significant increase in dressing percentage when diets were supplemented with copper sulfate. Barber et al. (1960a) noted an increase in dressing percentage in one experiment

but failed to confirm it subsequently. Castell and Bowland (1968a) reported an increase in dressing percentage in one experiment but in another experiment, a decrease in dressing percentage was observed. DeGoey et al. (1971) observed a slightly reduced dressing percentage for pigs fed supplemental copper. Feeding pigs supplemental copper as copper sulfate has recently been observed to result in a non-significant higher dressing percentage as compared with control animals (Myres et al., 1972). Wallace et al. (1966b) have earlier observed that copper supplementation did not significantly affect dressing percentage.

Barber et al. (1960a), Barber et al. (1961a) and Bekaert et al. (1967) reported that copper supplementation of the diet tended to decrease carcass length. Barber et al. (1961b) and Wallace et al. (1966b) reported that dietary copper supplementation did not significantly affect carcass length. Castell and Bowland (1968a) and DeGoey et al. (1971) observed a general tendency for pigs that received supplemental copper to have shorter carcasses.

Results presented by Barber et al. (1961a) and Allen et al. (1961) indicated that copper supplementation of the diet may increase backfat thickness of market pigs, but other investigators, Barber et al. (1960a, 1961b), Wallace et al. (1966b), Bekaert et al. (1967), Castell and Bowland (1968a) and Myres et al. (1972) failed to confirm this observation. DeGoey et al. (1971) observed a significant reduction in backfat thickness when supplemental copper was fed.

It has been generally observed that an increase in loin eye area results when pigs are fed diets supplemented with copper (Braude et al., 1962; Lucas et al., 1962; Castell and Bowland, 1968a and DeGoey et al. 1971).

Reports have appeared in the literature indicating that dietary copper supplements may influence porcine fat composition. Taylor and Thomke (1964) and Thomke and Taylor (1964) reported that the supplementation of diets fed to market pigs with 250 ppm copper resulted in a small but significant increase in the iodine number of the backfat. Bowland and Castell (1965) reported a greater incidence of soft fat in pigs fed copper supplemented diets. Bekaert et al. (1967) reported a similar trend. They observed a significant decrease in the percentage of stearic acid and a significant increase in the percentage of oleic acid present in the backfat of pigs fed copper supplemented diets as compared with control animals. Moore et al. (1968) reported that supplementation of pig diets with 250 ppm copper resulted in a slight increase in the percentage of 18:1 (oleic acid) and a slight decrease in the percentage of 18:0 (stearic acid) present in the backfat, with no change in the concentration of other constituent acids. There was also a 10°C decrease in the melting point of the backfat of pigs fed supplemental copper as compared with the control animals. Christie and Moore (1969) reported a lower melting point for backfat of pigs fed supplementary

copper. Higher amounts of unsaturated fatty acids have been found in the backfat of pigs fed supplementary copper (Elliot and Bowland, 1968, 1970).

SUPPLEMENTAL FORM OF COPPER

The most common form in which copper is added to swine diets is as copper sulfate ($CuSO_4 \cdot 5H_2O$). In an experiment to determine whether the copper (Cu++) or the sulfate ($SO_4^{=}$) radical was responsible for improvement in hog performance, Hawbaker *et al.* (1959) fed three diets, basal, basal + Na_2SO_4 or basal + $CuCl_2$. The pigs fed the diet supplemented with Na_2SO_4 grew at a slower rate and had a poorer efficiency of feed conversion than those fed the control diet, while those fed the diet supplemented with $CuCl_2$ grew faster and had a better feed conversion rate than the control animals. They concluded that the Cu++ radical was the factor responsible for improved performance.

Lucas *et al.* (1961) supplemented diets with $CuSO_4 \cdot 5H_2O$ in both a commercial and a highly purified form. Similar effects on rate of gain and efficiency of feed conversion were observed regardless of purity of the copper sulfate employed. They concluded that the effect was due to the copper radical and not to impurities in commercial grade copper sulfate.

Several forms of copper: copper sulfate ($CuSO_4 \cdot 5H_2O$), copper chloride ($CuCl_2$), copper sulfide (CuS), copper oxide (CuO), copper

carbonate ($CuCO_3$) and copper methionine have been used as supplements in practical swine diets. Early studies with rats have shown that anemic rats were unable to use the copper of copper sulfide or copper porphyrin, whereas the oxide, hydroxide, iodide, glutamate, glycerophosphate, aspartate, citrate, nucleinate, and pyrophosphate were readily utilized (Schultze et al., 1936). Barber et al. (1960b) and Barber et al. (1961a) reported that supplemental copper supplied as $CuSO_4 \cdot 5H_2O$ resulted in significant response in rate of gain and feed conversion in pigs while there was no response observed when the supplement was supplied as CuS. Bowland et al. (1961) indicated that pigs absorb copper from cupric sulfide much less efficiently than that from cupric sulfate. Bunch et al. (1964, 1965) reported that copper in the form of $CuSO_4 \cdot 5H_2O$, $CuCO_3$ or Cu-methionine when added to pig diets resulted in significantly faster and more efficient gains than were obtained from diets without supplemental copper.

It is clear that copper in several salt forms can be effective in promoting gain in pigs. There is, however, a reason for the common usage of $CuSO_4 \cdot 5H_2O$. Braude (1965) recommended that copper sulfate be used in preference to other copper salts because the compound has a bitter taste and pigs will refuse to consume rations containing copper in this form at dangerously high levels.

LEVEL OF COPPER

Numerous experiments have been conducted to determine the level of dietary copper supplementation required to give optimum response. Levels of supplemental copper used in such experiments have ranged from 0 to 1250 ppm. Maximum performance has been associated consistently with 200 to 250 ppm supplemental copper (Barber et al., 1957; Hawbaker et al., 1959, 1961; Bunch et al., 1960, 1961, 1964, 1965; Wallace et al., 1966; Castell and Bowland, 1968a; DeGoey et al., 1971; and Kline et al., 1971, 1972). In a recent study by Braude et al., (1970) three levels of supplemental copper: 170, 210 and 250 ppm were compared. No significant differences were reported in performance of pigs receiving copper at the different levels. In a review by Wallace (1967), he suggested that the most appropriate level of supplemental copper to feed is probably not less than 125 ppm and not more than 250 ppm.

COPPER METABOLISM

(a) Absorption

Following oral administration of radioactive copper to humans, the activity appears very rapidly in the blood (Bush et al., 1955). These workers concluded that this observation seemed to suggest that copper absorption occurs in the stomach or the upper small intestine or both in man. Thompsett (1940) earlier reported that

copper absorption takes place in the duodenum in man, while Sacks et al. (1943) showed that copper was absorbed mainly from the upper jejunum in dogs. In pigs, Bowland et al. (1961) using Cu^{64} observed that the principal sites of copper absorption are the small intestine and colon. However, the very rapid appearance of labelled copper in the urine suggested some absorption from the stomach. In rats, the stomach and, to a lesser extent, the duodenum are the major sites of absorption (Van Campen and Mitchell, 1965). Starcher (1969) indicated that the absorption site for copper in chicks is the duodenum. It has been reported that acid conditions favor copper absorption (Underwood, 1971); this could probably explain why the stomach and upper small intestine are the main absorptive sites since acid conditions do occur in these segments of gastrointestinal tract.

There is limited information on the mechanism of absorption of copper. Studies with mice by Gitlin et al. (1960) have indicated that two mechanisms may be involved in copper absorption: one which follows first-order kinetics and the other an enzymatic process. Using an in vitro technique, Crampton et al. (1964) were unable to show increased absorption of copper above a concentration of 1 ppm. This observation does not support the first-order kinetics portion of the hypothesis. A copper-binding protein has been demonstrated by Starcher (1969) in the mucosal cells of the duodenum of the chick which may play a role in copper absorption in this species.

Studies have shown that the amount of copper absorbed can be profoundly affected by other dietary factors and the chemical form, level and combinations of copper ingested. Using the changes in copper concentration in the liver or blood as a measure of relative absorption, it has been reported that type and level of protein in the diet (Beames and Lloyd, 1965; Combs et al., 1966; O'Donovan et al., 1966; Hanrahan and O'Grady, 1968), levels of other minerals notably molybdenum, zinc and cadmium (Kulwich et al., 1953; Allen et al., 1958; Miller et al., 1959; Barber, Braude and Mitchell, 1960; Suttle and Mills, 1966; Van Campen, 1969; DeGoey et al., 1971; Kline, Hays and Cromwell, 1971, 1972), the level of copper (Bass et al., 1956; Lucas and Calder, 1957; Ullrey et al., 1960; Allen et al., 1961; Braude et al., 1970) or form of copper (Barber et al., 1965; Van Campen, 1966; Van Campen and Scaife, 1967; Whanger and Weswig, 1971) can influence the assimilation of copper from the diet.

In normal human subjects, according to calculations of Cartwright and Wintrobe (1964), copper given by stomach tube was better absorbed than dietary copper to the extent of 30 percent. Even under optimum conditions, it has been shown that only 2 to 10 percent of the ingested copper is absorbed and retained by the pig (Bowland et al., 1961).

Little is known of the chemical forms in which copper exists in feedstuffs. Changes in these forms affecting availability must occur because fresh herbage is significantly less effective in

promoting body copper stores than hay or dried herbage of equivalent total copper content (Hartman and Bosman, 1970). In pasture herbage, much of the copper exists in bound form as water soluble organic complexes. Aqueous extracts of fresh herbage have been shown by Mills (1955) to contain a small proportion of their copper as free ions or as positively charged complexes. The greater part exists in the form of neutral or negatively charged complexes. When fed to copper-deficient rats these complexes induce a more rapid response, and greater liver copper storage, than does the feeding of equivalent amounts of copper as copper sulfate. As an outcome of these studies, Mills suggested that copper may be transported through the intestinal mucosa both as ionic copper and in the form of complexes such as those encountered in herbage. Seeleman and Baudissin (1954), (cited by Mills, 1955) observed that the sodium salt of a copper-allylthioureabenzoic acid complex is more effective in stimulating growth and hematopoiesis in the rabbit than is inorganic copper. Similar observations were later made by Kirchgessner and Wesser (1965) and Kirchgessner and Grassman (1970).

(b) Copper Transport in the Body

A schematic representation of the major metabolic pathways of copper in the body (Cartwright and Wintrobe, 1964; and Dowdy, 1969) is presented in Figure 1. From the intestine, copper moves into blood serum. In mammals, serum copper can be generally divided

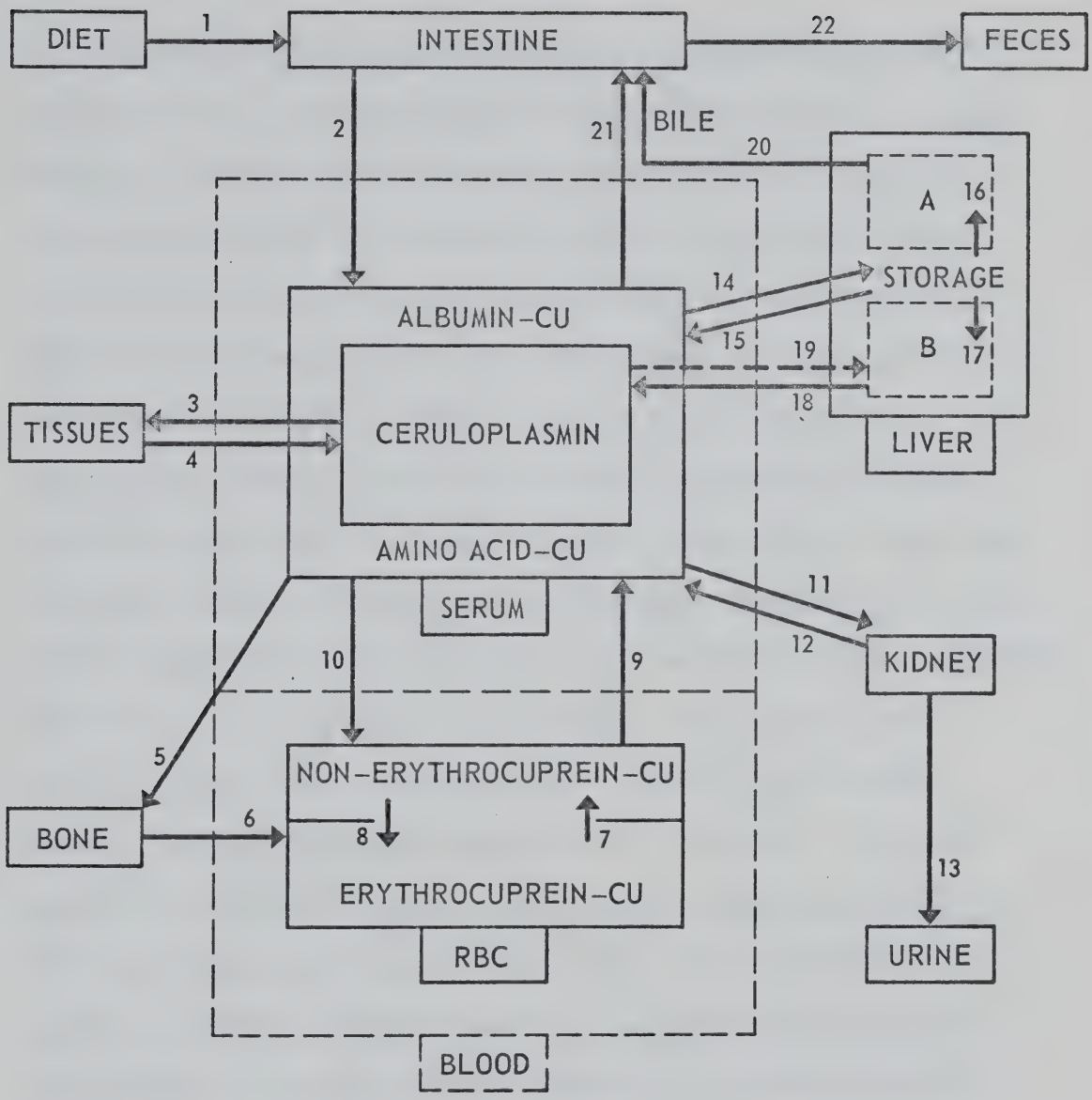

Fig. 1. Copper transport in the body

into two forms: a) direct-reacting copper (because it reacts directly in the serum with sodium diethyldithiocarbamate, a copper chelator); and b) indirect-reacting copper (because it will not react directly with the carbamate reagent). Radiocopper given orally appears rapidly in the direct reacting fraction and is believed to be associated with albumin (Bearn and Kunkel, 1954; Cartwright and Wintrobe, 1964). Later work by Neumann and Sass-Kortsak (1967) suggests that a small portion of the direct-reacting serum copper is bound to various amino acids. About two hours following oral dosage with radiocopper, peak activity in the direct reacting fraction is reached, followed by a rather precipitous decline in serum activity. Subsequently, serum radioactivity again increases, this time appearing in the indirect-reacting copper fraction which is believed to be, exclusively, the copper protein ceruloplasmin (Holmberg and Laurell, 1948; Bowland et al., 1961; and Neumann and Sass-Kortsak, 1967). When equilibrium is reached, about 93% of serum copper is in ceruloplasmin and 7% in the albumin- and amino acid-bound fractions (Neumann and Sass--Kortsak, 1967; and Dowdy, 1969). Gubler et al. (1953), using normal human subjects, reported that albumin-bound copper is the transport fraction in human system. Sternlieb et al. (1961) demonstrated that copper bound to ceruloplasmin is not exchangeable with ionic copper in vivo and is released only when the protein molecule is catabolized. The amino acid-bound fraction is thought to be involved with the movement of copper through membranes

(Dowdy, 1969). Evidence supporting this contention is that amino acids added *in vitro* facilitated copper uptake by liver slices under aerobic conditions (Harris and Sass-Kortsak, 1967).

From the blood, absorbed copper is widely distributed to the tissues but appears to accumulate mainly in the parenchymal cells of the liver and kidney. Many investigators, including Allen *et al.* (1958), O'Hara *et al.* (1960), Wallace *et al.* (1966b), Castell and Bowland (1968b) and Kline *et al.* (1972) have observed that liver is the major organ for copper storage. At least three important copper functions are associated with the liver: a) storage, b) ceruloplasmin synthesis (Owen and Hazelrig, 1966), and c) preparation of the metal for biliary excretion (Hazelrig and Owen, 1966).

(c) Copper Excretion

Biliary copper is emptied back into the intestine and is excreted as fecal copper. In fact the main route for excretion of absorbed copper is through the bile. In pigs, Bowland *et al.* (1961) have calculated that the bile could account for up to 40% of the total amount of copper excreted in the feces, while Cartwright and Wintrobe (1964) indicated a value of 80% biliary excretion of copper in human subjects. In their calculation, 16% of absorbed copper is emptied back into the intestine through the gut wall and only about 4% is excreted in the urine. Mahoney *et al.* (1955) indicated that pigs under normal conditions excrete only small quantities of copper via the kidneys or intestinal wall.

(d) Metabolic Role of Copper

Copper has various roles in the body, each probably related to some catalytic function. One of the first recognized functions of copper was in relation to hemoglobin formation (Hart et al., 1928). Despite an adequate supply of iron, copper is required to prevent anemia. At least three obvious possibilities are apparent for the role of copper in hematopoiesis. First, copper could facilitate iron absorption. Second, copper could be stimulatory to the enzymes in the heme or globin biosynthetic pathway(s) or both. Third, copper could be involved in the mobilization of stored iron, preparative to the incorporation of iron into the hemoglobin molecule (Dowdy, 1969). Data concerning the role of copper in iron absorption are conflicting with some workers reporting no effect (Schultze, 1940), while others report that copper increases iron absorption (Chase et al., 1952). In a review, it was suggested that copper affects iron absorption in an indirect manner (Matrone, 1960). Thus in copper deficiency, when hemoglobin synthesis is decreased, iron accumulates in the body (Smith and Ellis, 1947; Lahey et al., 1952). This would stimulate an iron load in the body and would signal the absorptive sites to block further iron absorption. When copper is added back to the diet, hemoglobin synthesis would be initiated, body iron stores would be reduced, and the absorptive sites would begin to function (Dowdy, 1969). Copper is apparently neither an activator nor an inducer for the heme biosynthetic pathway, since the enzymes

actually show greater activity under conditions of copper deficiency (Lee et al., 1968). Mortson and Allen (1967) remarked that the most probable role of copper in hemoglobin formation is its involvement in release of iron stores from the liver.

In addition to dietary anemia, copper deficiency has resulted in certain bone abnormalities in swine. Ad libitum feeding of a whole milk diet to young pigs led to a progressive loss of use of the hind-, then fore-legs (Teague and Carpenter, 1951). Supplemental copper at a rate of 2 mg/pig/day appeared capable of arresting the development of the symptoms. Administration of copper and iron resulted in a degree of reversal in the degenerative process but supplementing with vitamins A and D had no effect. Similar observations were reported by Lahey et al. (1952) but a smaller proportion of deficient animals developed the symptoms, possibly as a result of a higher level of dietary iron.

Investigations of the effects of low levels of dietary copper revealed a marked diminution of osteoblastic activity in bone sections from deficient pigs (Follis et al., 1955). The authors remarked on the similarity of the symptoms to those of scurvy and reported that both copper and ascorbic acid seem to have a unique property in common, i.e. ability to interfere specifically with the functional activity of the osteoblasts while not affecting the integrity of cartilage cells. The mechanism of copper in the process of bone formation has not been identified.

Another role of copper in the body concerns energy metabolism.

Copper has been shown to be a constituent of cytochrome oxidase (Schultze, 1941), the terminal oxidase in the electron transport mechanism from which high energy phosphate bonds are derived. The activity of this enzyme is markedly reduced in the heart and liver of copper-deficient swine (Gubler et al., 1957). Gallagher and coworkers (1956) demonstrated a significant fall in cytochrome oxidase activity of the brain, as well as of the heart and liver of rats in copper deficiency.

Copper has been shown to be associated with formation of aortic elastin (O'Dell et al., 1961; Starcher et al., 1964; and Hill, 1969). Elastin is a protein which provides elasticity in blood vessels, whereas collagen imparts strength and rigidity (Bendall, 1964). A copper deficiency not only reduced by 50 percent the concentration of elastin in chick aorta (O'Dell et al., 1966), but in swine reduced tensile strength of elastin as well (Carnes et al., 1961). Recent evidence shows that copper deficiency interferes with both elastin and collagen (Chou et al. 1968).

EFFECTS OF DIETARY COMPONENTS ON RESPONSE TO SUPPLEMENTAL COPPER

(a) Mineral Balance

i) Molybdenum

The effects of molybdenum on copper utilization and toxicity are very conflicting, especially with regard to various animal species. In ruminants, molybdenum is effective in counteracting

copper toxicity. Dick (1953, 1954, 1955) and Wynne and McClymont (1955) demonstrated that liver copper stores of sheep could be reduced by feeding adequate molybdenum. Reports with rats (Comar et al., 1949) and pigs (Kulwich et al., 1953; Pond and Smith, 1967) suggest that, in contrast to ruminants, the response of non-ruminants to copper is less dependent on the molybdenum content of the diet. Kline et al. (1971) indicated that molybdenum added at 25, 50, 100 and 200 ppm to pig rations had no significant effect on performance, hematology or tissue copper stores of pigs fed 150, 200 or 250 ppm dietary copper. Conversely, Dowdy and Matrone (1968a,b) reported that copper offered as a copper -molybdenum complex in the diet was less available to baby pigs than copper offered as copper sulfate or copper citrate for ceruloplasmin synthesis, indicating a copper-molybdenum antagonism in piglets. However, Whanger and Weswig (1970) failed to demonstrate copper unavailability by feeding a copper-molybdenum complex to rats.

ii) Zinc, cadmium, iron, silver and mercury

Several workers have shown that adding large quantities of zinc to the diet of rats prevents an otherwise adequate dietary copper supply from meeting the rats' requirements for copper (Smith and Larson, 1946; Grey and Ellis, 1950; Van Reen, 1953; Hill et al., 1963). Cox and Hale (1962) were, however, unable to demonstrate a similar antagonism in the pig. Ritchie et al. (1963) have reported a protective effect of a 100 ppm zinc supplement against the toxic effects of 250 ppm dietary copper on pigs.

Allen et al. (1958), Barber, et al. (1960) and Hanrahan and O'Grady (1968) indicated that liver copper stores of pigs fed 250 ppm supplemental copper were lowered by additional dietary zinc, ranging from 125 to 250 ppm. Similarly, DeGoey et al. (1971) reported that supplemental zinc and iron reduced hepatic storage of copper in pigs fed 250 ppm dietary copper. However, results presented by Kulwich et al. (1953), Gipp et al. (1967) and Kline et al. (1972) failed to demonstrate that dietary zinc protected pig tissues from high copper levels. Nonetheless, the thesis that zinc is antagonistic to copper seems well documented (Duncan et al., 1953; Magee and Matrone, 1960; Hill and Matrone, 1962; Hill et al., 1964; Van Campen, 1966; Van Campen and Scaife, 1967; Carrico and Deutsch,1970; Whanger and Weswig, 1971). The mechanism of this interaction has not been fully explored.

It has been observed that cadmium acts in a manner similar to zinc in copper antagonism (Hill et al., 1963; Hill et al., 1964; and Van Campen, 1966). A mechanism of interaction has been proposed for both zinc and cadmium. Starcher (1969) first identified a copper-containing protein of 10,000 molecular weight in the chick intestine and suggested that cadmium and zinc antagonize copper absorption by displacing copper from this duodenal protein. Recently, Evans et al. (1970a) examined the metabolism of copper and zinc at the molecular level and demonstrated that the two elements are bound to a single 10,000 molecular weight protein in the duodenum, liver and kidney of the rat. The characteristics of the

identified protein in rat and chick are similar to that of metallothionein previously isolated from equine and human kidney by Kagi and Vallee (1960, 1961) and Pulido et al. (1966). Evans et al. (1970) further indicated that the major fraction of soluble copper from both bovine duodenum and liver is associated with a protein similar to metallothionein and that the second fraction of soluble copper is associated with a protein similar to cytocuprein which has been described by Carrico and Deutsch (1969, 1970). Kagi and Vallee (1961) and Pulido et al. (1966) demonstrated that the metal ions of metallothionein are bound to mercapto groups. Since cadmium and zinc form stable mercaptides, Evans et al. (1970a,b) suggested that these elements displace copper from the sulfhydryl binding sites on metallothionein.

Several investigators have shown that the addition of copper to pig diets causes a depression in liver iron stores (Cassidy and Eva, 1958; Bunch et al., 1963; and Ritchie et al., 1963). Kainski et al. (1967) reported that high dietary levels of iron reduced liver copper stores in pigs fed excess copper, while Kline et al. (1972) failed to obtain any protection against increased copper accumulation in the liver of pigs fed high levels of copper and iron.

Silver and mercury may also alter copper metabolism (Hill et al., 1964; Van Campen, 1966; and Whanger and Weswig, 1970).

Generally, the Cu^{+} ion forms tetrahedral complexes and the Cu^{2+} ion forms square coplanar complexes; both ions have a preferred coordination number of 4. Theoretically, any ion

capable of attaining these chemical characteristics can act as a copper antagonist. Since Cd^{2+}, Zn^{2+} and Ag^{+} ions can attain such characteristics, they could be expected to act antagonistically to copper. Data presented by Hill et al. (1963), Hill et al. (1964) and Whanger and Weswig (1970, 1971) support this hypothesis.

(b) Level and Type of Protein

i) Level of protein

Reports regarding the effects of level of protein on the response to dietary copper supplementation are conflicting. Bunch et al. (1961) reported that the response to copper was similar in corn-soybean meal and corn-fishmeal diets containing either 16% or 22% protein. King (1964) observed that in barley-fishmeal diets with a low (14.5%) protein level, copper supplementation resulted in improved rate of gain which was not observed in the high protein (18.1%) diet. Similarly, Beames and Lloyd (1964) observed that copper supplementation of swine diets containing a low level of soybean meal (6%) resulted in improved performance, while supplementation of diets containing a high level of soybean meal (23%) did not affect rate of gain, but tended to reduce feed consumption. Combs et al. (1966) observed that levels of protein (14 or 22%) as either soybean meal or casein did not affect the response to copper supplementation of the diet. Castell and Bowland (1968a) indicated that the beneficial effects of copper

supplementation were more evident when diets of relatively high protein content were fed. Hanrahan and O'Grady (1968) observed that the performance of pigs fed a low protein diet supplemented with copper was significantly reduced while that of pigs fed a high protein copper supplemented diet was only slightly reduced.

ii) Type of protein

Fishmeal is a common protein supplement in Great Britain, where the initial studies of copper supplementation of swine diets were carried out. Early studies in North America, where soybean meal is the protein supplement generally used, did not demonstrate the same response. This indicated that the type of protein supplement, animal versus vegetable, used in the diet might affect the response to dietary copper supplementation.

Allen et al. (1961) reported that when copper at a level of 250 ppm was added to diets containing either fishmeal or dried skim milk as protein sources, response in terms of average daily gain, feed conversion and feed intake, was greater in diets supplemented with dried skim milk than in diets supplemented with fishmeal. Braude et al. (1961) observed greater response to copper supplementation of diets containing fishmeal than in diets containing soybean meal, whereas Lucas et al. (1962) did not obtain any difference in the effect of copper supplementation of barley-fishmeal diets as compared with soybean meal diets as long

as dietary protein was adequate. Similar observations have been made by Combs et al. (1966) and O'Donovan et al. (1966). However, Castell and Bowland (1968a) and Drouliscos et al. (1969) reported that the beneficial effects of copper were more evident in pigs fed diets supplemented with fishmeal than in pigs fed diets containing soybean meal.

RAPESEED MEAL AS A PROTEIN SOURCE

Most of the early work concerning copper supplementation in swine rations either involved fishmeal or soybean meal as protein sources. In recent years in Canada, considerable attention is being given to the utilization of rapeseed meal in swine diets.

Bowland (1966) reviewed extensively the feeding value of rapeseed meal for swine. Schuld and Bowland (1968) reported that 8% rapeseed meal in the starting and growing diets for pigs, when replacing soybean meal on an isonitrogenous basis, had no depressive effect on feed consumption, but did depress growth performance and efficiency of feed utilization. Bayley et al. (1969) found no effect on performance during the finishing phase by adding 11% rapeseed meal to replace an equivalent level of protein from soybean meal. Elliot and Bowland (1970), using copper supplemented diets, observed that pigs fed barley-fishmeal and barley-soybean meal diets performed better in terms of weight gain and feed conversion than those fed barley-meat meal and barley-rapeseed

meal diets, but there were no interactions between level of copper and protein source. Saben and Bowland (1971) reported that 8% rapeseed meal, when replacing soybean meal on an isonitrogenous basis, fed to sows both in the gestation and lactation periods for two reproductive cycles, had no significant effects on feed conversion efficiency, gestation weight gains or lactation weight losses.

In early studies with solvent-extracted rapeseed meal of Brassica campestris type and free of the enzyme myrosinase, Bell (1965) obtained no reduction in energy digestibility of the diet, when the meal was fed to growing and finishing pigs. The addition of ground rapeseed screenings added as the source of the enzyme myrosinase, caused a depression in the digestibility coefficients of energy and protein.

The growth-inhibiting properties of rapeseed meal appear dependent upon hydrolysis of glucosinolates (thioglucosides) into isothiocynates (2-hydroxy-3-butenyl) and oxazolidinethione. The hydrolysis is affected by the enzyme myrosinase, normally present in unheated rapeseed (Greer, 1956; Kjaer, 1960; Virtanen, 1965). Several bacterial species in the gastrointestinal tract have been found to possess enzymes capable of hydrolyzing thioglucosides (Bell and Belzile, 1965).

Bell and Belzile (1965) indicated that commercially produced enzyme-free rapeseed meal, containing unhydrolyzed glucosinolates, is free of most of the undesirable properties if myrosinase is not

reintroduced by other dietary means or by intestinal bacteria. Lodhi et al. (1970) fed diets with and without myrosinase, and containing up to 30% rapeseed meal, and showed no effect of dietary rapeseed meal on the metabolizable energy value of chick diets.

GENERAL EXPERIMENTAL

MANAGEMENT OF EXPERIMENTAL ANIMALS

All pigs used in this work were farrowed at The University of Alberta Edmonton Research Station and were of Hampshire X Yorkshire breeding.

The standard management practices followed in The University of Alberta Swine Research Unit were followed. All pigs were identified by ear notch at birth and "black" teeth were cut off. For prevention of anemia, a 2 ml intramuscular injection of Imposil-200[1] (an iron dextran compound containing 100 mg of injectable iron/ml) was administered to each animal at approximately 4 days of age. Male pigs were castrated at 10 days of age, and all pigs were weaned at three weeks and fed a prestarter ration containing approximately 20% crude protein. Between five and six weeks of age, pigs were treated for ascarids, using a piperazine derivative[2] and for skin parasites, using lindane[3] spray. Vaccination against

---

[1] Fisons of Canada Ltd., 26 Prince Andrew Place, Don Mills, Ontario.

[2] Western Brand Products Ltd., Edmonton, Alberta. 616 mg/100 kg feed.

[3] Gamma-benzene hexachloride supplied by Franklin Laboratories Ltd., Calgary, Alberta

erysipelas using a subcutaneous injection of a commercial bacterin[1] was carried out when the pigs were approximately 8 weeks old.

All experimental pigs were paired in concrete-floored pens until they attained 40 kg weight when they were separated and fed individually. Each pen measured 0.58 x 1.17 m and was equipped with a feeding stall and an automatic waterer. Both experiments were conducted in the same building at an approximate temperature of 65°F.

Diets were mixed and bagged at The University of Alberta feed mill and were stored in the barn where the pigs were on experiment.

METABOLISM STUDIES

Metabolism crates as used previously by Hussar (1958), measuring 73 cm high, 116 cm deep and 41 cm wide, situated approximately 60 cm above the concrete floor were used in the present study. They were constructed of angle iron and galvanized sheet metal. The floor of the crate consisted of a rolled expanded metal sheet with 18 x 45 mm mesh. Beneath this mesh was a 6 x 6 mm aluminium screen above a collection tray which had a gradual slope from the edges to a central drain hole. Urine was collected in a container situated under the tray. Feces were, to a large extent,

---

[1] Agricultural Division, Chas. Pfizer and Co., Inc., New York, N.Y.

collected on the expanded metal sheet, and the remainder were forced through the perforated floor and retrieved from the collection tray. Feed and water containers were attached at the front of the metabolism crate.

COLLECTION AND HANDLING OF SAMPLES

(a) Feed Samples

Samples of feed for determination of dry matter, gross energy and crude protein were obtained at the time of mixing with the aid of a seed sampler[1]. One sample was taken from each bag in the mix. The samples were pooled prior to sampling for analyses.

(b) Fecal Samples

Feces were collected each morning during the metabolism periods. Each day's collection was placed in a labelled plastic bag and stored at 3°C. After final collection, total feces collected from each pig were thoroughly mixed and weighed. The fecal samples were then dried in a forced air oven[2] for 72 hours at 60°C, allowed to equilibrate with the air for a further 24 hours before final weighing. Subsequently, the samples were ground in a C and N laboratory mill[3], to pass through a 2 mm mesh screen and

---

[1] Seedboro Equipment Co., Chicago, Illinois, U.S.A.

[2] Style v31, Despatch Oven Co., Minneapolis, Minn., U.S.A.

[3] Size 8, Christy and Norris Ltd., Chelmsford, England.

representative samples retained for later analyses.

(c) Urine Samples

Collections of urine were made at the same time the feces were collected. Volume was measured and recorded at the time of collection. Aliquots of urine were placed in jars, sealed and stored at 3°C such that a total volume of approximately 250 ml was collected over the three day period.

(d) Liver and Kidney

The livers and kidneys of the test animals were obtained from the Swift Canadian packing plant in Edmonton shortly after slaughter. The samples were stored in sealed plastic bags in the freezer at -16°C prior to analyses.

(e) Fat and Muscle

A pork chop from the vicinity of the 7th and 8th ribs of the left side was obtained from each pig. This chop of about 150 g fresh weight consisted of a strip of backfat, approximately 2.5 cm wide, and the longissimus muscle. Chops were stored in sealed plastic bags in the freezer at -16°C prior to analyses.

CHEMICAL ANALYSES

(a) Dry Matter

i) Urine:

Approximately 200 ml of urine was weighed into an aluminium foil dish and placed in a freeze-dryer[1] for 48 hours. Urine dry matter was computed on the basis of dry weight.

ii) Liver, kidney and muscle:

Weighed samples of tissue were dried in a freeze-dryer for 48 hours and transferred to a forced-air oven for another 24 hours at 110°C. The samples were then cooled in a dessicator until constant weight was obtained.

(b) Nitrogen

The nitrogen (N) content of feed and feces was determined by the Kjeldahl method of analyses (AOAC, 1960). A commercial Kel-Pak[2] was used to provide the required amount of catalyst for the acid digestion. The ammonia ultimately produced was retained in a 4% boric acid solution and titrated directly with standard H_2SO_4.

---

[1] Repp Sublimator Model SRC42. Division of Virtis Co., Inc., Gardiner, N.Y. 12525, U.S.A.

[2] Matheson Scientific, East Rutherford, New Jersey. Supplies HgO, K_2SO_4 and $CuSO_4$.

(c) Gross Energy

Gross energy of feed, feces and urine was determined using a Parr Oxygen Bomb Calorimeter[1] equipped with a Brown Electronik recorder[2].

(d) Lipid Analysis

i) Lipid extraction

The method of Folch et al. (1957) was used to extract fat samples. A 1 g sample was homogenized with 20 ml of $CHCl_3$:CH_3OH (2:1) in a Virtis "23" homogenizer[3]. The homogenizing vessel was surrounded by ice.

ii) Preparation of methyl esters

Methyl esters were prepared from the extracted lipids by transesterification according to a slight modification of the method of Morrison and Smith (1964) for triglycerides; n-pentane replaced benzene in this method. The lipid extracts were first filtered through anhydrous Na_2SO_4 to remove any water, the solvent removed under N_2 on a flash evaporator[4] and approximately 50 mg

---

[1] Parr Instrument company. Moline, Illinois, U.S.A.

[2] Minneapolis - Honeywell Regulator company, Philadelphia, Pennsylvania, U.S.A.

[3] The Virtis company, Yonkers, N.Y., U.S.A.

[4] Rinco Instrument Co., Inc., Greenville, Illinois, U.S.A.

of the lipid so obtained transferred under N_2 to a 150 x 25 mm screw cap culture tube[1] for methanolysis.

iii) Gas-liquid chromatography

Each sample of methyl ester was chromatogrammed in duplicate using a Bendix Gas Chromatograph-2500[2] equipped with a flame ionization detector. Separation of the methyl esters was achieved on a 366 cm long and 0.3 cm inner diameter glass column packed with 15% diethylene glycol succinate on 100-200 chromosorb W. The following isothermal conditions prevailed: column temperature 200°C, injector and detector temperature 230°C, helium flow rate 50 ml per minute. The suppression was set at 1000 and slope sensitivity at 8.

iv) Identification of acids

Individual fatty acids were identified by comparing their retention times with the retention times of pure fatty acid methyl esters. The following fatty acids were identified: 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:1[3]. Several other minor components (less than 0.5%) were present, but these were not identified. The acids 14:0, 16:0, 16:1, 18:0, 18:1 and 18:2 together accounted for more than 98% of the fatty acids evaluated

---

[1] Cat. No. 9826. Corning Glassworks, Fisher Scientific, Edmonton

[2] Bendix International Operations, 605 Third Avenue, New York, N.Y. 10016, U.S.A.

[3] 14:0 myristic acid; 16:0 palmitic acid; 16:1 palmitoleic acid; 18:0 stearic acid; 18:1 oleic acid; 18:2 linoleic acid; 18:3 linolenic acid; 20:1 gadoleic acid.

in the samples. The RSM might contain traces of erucic acid (22:1) from residual rapeseed oil; however, no erucic acid determination was carried out in these studies.

v) Methods of calculation

1. Calculation of peak areas:

Peak areas were obtained with the aid of a Vidar 6300 digital electronic integrator[1] attached to the recorder[2].

2. Calculation of percent composition:

Correction factors for detector response were obtained by chromatogramming each day a standard sample of known weight percent composition which approximated the composition of porcine depot fat. The percentage composition of the standard sample was determined from the peak areas and correction factors obtained by comparing these figures with the known weight percent composition of the sample. All correction factors derived for a given acid on a given column were averaged and applied to chromatograms run on that column to arrive at corrected peak areas. The corrected areas were totalled and the individual areas expressed as a percentage of the total to arrive at weight percent composition.

(e) Copper determination

The method of analysis for copper content of pig tissues

---

[1] Vidar Autolab., 77 Ortega Ave., Mountain View, Calif. 94040, U.S.A.

[2] Microcord Model 44. Photovold Corporation, 1115 Broadway New York, N.Y. 10010, U.S.A.

was based on the procedure described by Middleton and Stuckey (1953) and reviewed by Anderson (1972). All glassware was thoroughly cleaned with hot detergent solution, rinsed and soaked overnight in 1:1 nitric acid. After soaking, glassware was rinsed with hot tap water, distilled water and demineralized water, then dried at 110°C. Before use, the glassware was stored separately from other containers and protected from contamination. Only demineralized water was used throughout the analysis. To obtain demineralized water, distilled water was passed through a Bantam Demineralizer[1] equipped with a standard cartridge.

Each dry sample was thoroughly ground to obtain as homogenous a sample as possible. Dried samples weighing 1 g were placed in porcelain evaporating dishes and ashed in a controlled-temperature muffle furnace[2]. The furnace was set at an initial temperature of 250°C for six hours, increased to 400°C for a further four hours and finally adjusted to 500°C for a total ashing period of 24 hours. The samples were then cooled and 5 ml of concentrated nitric acid were added to the ashed sample. After standing for approximately one hour, the samples were transferred to 100 ml volumetric flasks and diluted to volume with deionized water.

Copper concentration was measured by direct aspiration into a model AA-4 atomic absorption spectrometer[3].

---

[1] Barnstead Still and Sterilizer Co., Boston 31, Mass., U.S.A.

[2] Lindberg Hevi-duty. Fisher Scientific Co. Ltd., 8505 Devonshire Road, Montreal, Quebec.

[3] Atomic Absorption Spectrometer, 23-31 Islington Street, Melbourne, N.S.W., Australia.

STATISTICAL ANALYSES

Analysis of variance was used in statistical analyses of the data. Because of the two additional treatments of supplementary iron and manganese for pigs receiving SBM or RSM alone in Experiment 1, it was necessary to perform two separate sets of analyses. The first analyses consisted of protein (n = 3), copper (n = 5), sex (n = 2) with four pigs per treatment group; while the second analyses consisted of protein (n = 2), mineral (n = 7), sex (n = 2) with four pigs per treatment group. One set of analyses was made in Experiment 2 consisting of protein (n = 3), mineral (n = 4) and sex (n = 2) with four pigs per treatment group. All sources of variation except pigs were considered as fixed. There were no first or second order interactions in experiments. No discussion of three factor or higher interactions will be made.

Computation was made on an IBM 360/67 computer using Library program CS017, University of Alberta (1968) modified by Weingardt (1972)[1]. Differences between three or more treatment means were compared using Duncan's multiple range test (Steel and Torrie, 1960). Missing data were replaced by the mean of that treatment group with the consequent loss of one degree of freedom in the error term per replacement (Steel and Torrie, 1960).

---

[1] Ray Weingardt - personal communication.

EXPERIMENTAL OUTLINE

In Experiment 1, started in the spring, 1971, 76 early-weaned pigs, equalized as to sex and averaging 6 kg in weight, were allotted to 19 treatment groups of 4 pigs each (Table 1). The crossbred Hampshire-Yorkshire pigs were allotted at random within sex and were fed a SBM-supplemented diet, a RSM (*Brassica napus* cultivar Bronowski, low glucosinolate)-supplemented diet or a diet supplemented with 50% each of protein from SBM and RSM (Table 2). Within each dietary protein source, further sub-division was made on the basis of level of supplemental copper (0, 125, or 200 ppm), presence or absence of supplemental iron and manganese and withdrawal of copper at 40 kg liveweight or continuous copper supplementation to the end of the experiment.

In Experiment 2, 48 Hampshire-Yorkshire crossbred pigs of 10.5 kg average initial weight, equalized as to sex were randomly allotted in groups of four in the spring, 1972, to 12 treatments (Table 3). Protein and copper supplementation were as in Experiment 1, except that RSM from *B. campestris* cultivar Span seed was used. This is a low erucic acid (LEAR) type of rapeseed. The same dietary formulations (Table 4) of approximately 14.4% crude protein were fed throughout with no adjustment in protein or other nutrients. All diets contained 50 ppm zinc which meets recommended requirements (NAS-NRC, 1968). Some of the diets supplemented with 200 ppm copper were further supplemented with 200 ppm zinc.

In both experiments, pigs were fed *ad libitum* and had free

Table 1: ALLOTMENT OF PIGS IN EXPERIMENT 1

Treatment Number	Source of Supplemental Protein	Level of added Copper (ppm)		Supplemental iron and manganese
		to 40 kg	40 kg to market	
1	SBM [1]	0	0	0
2	SBM	125	0	0
3	SBM	125	125	0
4	SBM	200	0	0
5	SBM	200	200	0
6	SBM	0	0	+ [3]
7	SBM	200	200	+
8	SBM + RSM [2]	0	0	0
9	SBM + RSM	125	0	0
10	SBM + RSM	125	125	0
11	SBM + RSM	200	0	0
12	SBM + RSM	200	200	0
13	RSM	0	0	0
14	RSM	125	0	0
15	RSM	125	125	0
16	RSM	200	0	0
17	RSM	200	200	0
18	RSM	0	0	+
19	RSM	200	200	+

[1] SBM = soybean meal (44% protein)

[2] RSM = rapeseed meal (B. napus, cultivar Bronowski; 37% protein)

[3] To provide 41 ppm iron (Fe) as ferrous sulfate and 81 ppm manganese (Mn) as manganese sulfate

Table 2: FORMULATION AND COMPOSITION OF BASAL DIETS FOR PIGS IN EXPERIMENT 1

Source of Supplemental Protein	SBM		SBM + RSM		RSM	
Period	start to 40kg	40kg to market	start to 40kg	40kg to market	start to 40kg	40kg to market
Ingredients						
Wheat (ground)	47.0	10.0	59.7	20.0	72.4	30.0
Barley (ground)	30.0	77.0	15.0	65.9	-	54.7
Soybean meal (44%)	20.0	10.0	10.0	5.0	-	-
Rapeseed meal (37%)	-	-	12.3	6.1	24.6	12.3
Ground limestone	1.0	1.0	1.0	1.0	1.0	1.0
Calcium phosphate[1]	1.1	1.1	1.1	1.1	1.1	1.1
Iodized salt	0.5	0.5	0.5	0.5	0.5	0.5
Trace mineral - vitamin mix[2]	0.4	0.4	0.4	0.4	0.4	0.4
Composition (calculated)						
Digestible Energy[3] kcal/kg	3289	3128	3281	3129	3274	3149
DE supplied by SBM kcal/kg			330	165		
DE supplied by RSM kcal/kg			344	161		
Crude protein .....%	18.4	14.3	18.5	14.4	18.5	14.5
Crude protein from SBM..%			4.5	2.3		
Crude protein from RSM..%			4.6	2.3		
Lysine .....%	0.86	0.69	0.82	0.66	0.78	0.64
Methionine and Cystine..%	0.56	0.46	0.55	0.45	0.54	0.44
Calcium .....%	0.73	0.72	0.75	0.73	0.79	0.75
Phosphorus .....%	0.63	0.61	0.66	0.62	0.68	0.64

[1] The calcium phosphate contained 18.5% Ca and 20.5% P.

[2] To supply 440 mg riboflavin, 880 mg calcium pantothenate, 2 g niacin, 2.2 g choline chloride, 15 mg folic acid, 1 mg vitamin B_{12}, 500,000 I.U Vitamin A, 66,000 I.U Vitamin D_2, 1,000 I.U Vitamin E and 113 mg cobalt per 100 kg diet. It also supplies 50 ppm zinc, 10 ppm copper, 112 ppm iron and 37 ppm manganese.

[3] Digestible Energy = DE

Table 3: ALLOTMENT OF PIGS IN EXPERIMENT 2

Treatment Number	Source of Supplemental Protein	Level of added Copper (ppm)	Level of added Zinc (ppm)
1	SBM[1]	0	0
2	SBM	125	0
3	SBM	200	0
4	SBM	200	200
5	SBM + RSM[2]	0	0
6	SBM + RSM	125	0
7	SBM + RSM	200	0
8	SBM + RSM	200	200
9	RSM	0	0
10	RSM	125	0
11	RSM	200	0
12	RSM	200	200

[1] SBM = soybean meal (44% protein)

[2] RSM = rapeseed meal (B. campestris, cultivar Span, 37% protein)

Table 4: FORMULATION AND COMPOSITION OF BASAL DIETS FOR PIGS IN EXPERIMENT 2

Source of Supplemental Protein	SBM	SBM + RSM	RSM
Ingredients			
Wheat (ground)	10.0	20.0	30.0
Barley (ground)	77.0	65.9	54.7
Soybean meal (44%)	10.0	5.0	-
Rapeseed meal (37%)	-	6.1	12.3
Ground limestone	1.0	1.0	1.0
Calcium phosphate[1]	1.1	1.1	1.1
Iodized salt	0.5	0.5	0.5
Trace mineral vitamin mix[2]	0.4	0.4	0.4
Composition (calculated)			
Digestible Energy[3] kcal/kg	3128	3129	3149
DE supplied by SBM kcal/kg		165	
DE supplied by RSM kcal/kg		161	
Crude protein .....%	14.3	14.4	14.5
Crude protein from SBM..%		2.3	
Crude protein from RSM..%		2.3	
Lysine .....%	0.69	0.66	0.64
Methionine and Cystine..%	0.46	0.45	0.44
Calcium .....%	0.72	0.73	0.75
Phosphorus .....%	0.61	0.62	0.64

[1] The calcium phosphate contained 18.5% Ca and 20.5% P.

[2] To supply 440 mg riboflavin, 880 mg calcium pantothenate, 2 g niacin, 2.2 g choline chloride, 15 mg folic acid, 1 mg vitamin B_{12}, 500,000 I.U vitamin A, 66,000 I.U vitamin D_2, 1,000 I.U vitamin E, and 113 mg cobalt per 100 kg diet. It also supplies 50 ppm zinc, 10 ppm copper, 112 ppm iron and 37 ppm manganese.

[3] Digestible Energy = DE

access to water. Pig weights and feed consumption were recorded once weekly.

Metabolism studies were conducted in both experiments. In Experiment 1, 42 pigs, 21 each of barrows and gilts, were selected for digestibility and retention studies on the basis of weight, mineral and protein treatment groups. Twenty-four pigs, 2 (1 barrow and 1 gilt) from each of the 12 treatments were selected for similar studies in the second experiment. Metabolism cages as described under 'General Experimental' were used.

Because of space limitations, pigs in both experiments were marketed at an average liveweight of 77 kg. This is below the usually recommended weight of market pigs. However, the weight was sufficiently heavy to allow the carcasses to fall in the normal range for routine grading by Canadian standards and assignment of a grade index under the "Hog Carcass Valuation System".

ROP CARCASS EVALUATION

In Canada, measurements of carcass quality for pigs on Record of Performance (ROP) for swine are based on the revised ROP standards of the Canada Department of Agriculture. The carcass ROP score provides an indirect estimate of the combined percent yield of the four lean cuts of a carcass and is calculated thus:

$$ROP = 51.68 - 3.234(A) + 1.0381(B) + 0.485(C) + 11.76(D) \pm E$$

where: A = Total backfat (sum of shoulder back and loin, in)

B = Loin area (area of loin at 14th rib, sq in)

C = Ham/carcass (%)

D = Area lean of ham/ham weight (sq in/lb)

E = Sex correction factor (+ 1.1 percent for barrows;
- 1.1 percent for gilts).

RESULTS AND DISCUSSION

GROWTH PERFORMANCE

(a) Effects of Copper Supplements

The mean values for feed intake, daily gain and efficiency of feed conversion for Experiment 1 are presented in Tables 5 and 6. Feed intake, daily rate of gain and efficiency of feed utilization were not significantly affected by dietary copper during the starting period to 40 kg liveweight. At this weight, copper supplements were removed from the diets of a portion of the pigs. For the overall experiment, daily feed intake and efficiency of feed conversion did not differ among any of the treatment groups. However, pigs that received 200 ppm copper throughout the experiment gained 0.66 kg per day which was non-significantly more rapid than the gain for pigs in any other treatment. The average improvement was approximately 8% over pigs receiving no supplemental copper. These results agree with those reported in reviews by Braude (1965) and Wallace (1967) and research reports by Barber et al. (1955), Hawbaker et al. (1961), Castell and Bowland (1968a) and Young et al. (1970) who observed improved performance in pigs with supplemental dietary copper at

Table 5: EXPERIMENT 1. EFFECTS OF DIETARY COPPER LEVELS AND SUPPLEMENTAL PROTEIN SOURCES[1] ON PERFORMANCE OF GROWING GILTS AND BARROWS[2]

	Copper Levels (ppm)					Protein Source			Sex	
	0	125 to 40kg	125	200 to 40kg	200	SBM	SBM + RSM	RSM	M	F
Number of pigs	12	12	12	12	12	20	20	20	30	30
Av. initial weight ...kg	5.6	5.9	5.9	6.4	6.3	5.8	5.9	6.3	6.0	5.9
Av. final weight .....kg	77.8	76.7	76.8	76.5	76.5	76.2	77.3	77.2	77.2	77.1
Growth Data										
Initial - 40 kg										
Daily feed ....kg	1.12	1.11	1.13	1.15	1.28	1.14	1.11	1.18	1.08	1.17
Daily gain ....kg	0.44	0.49	0.45	0.49	0.50	0.47	0.47	0.46	0.47	0.46
Feed/kg gain ..kg	2.54	2.27	2.50	2.34	2.56	2.42	2.37	2.56	2.30	2.54
40 kg - 77 kg										
Daily feed ....kg	2.99	2.91	2.73	2.99	2.98	2.98	2.78	3.02	2.98	2.79
Daily gain ....kg	0.78	0.77	0.78	0.73	0.82	0.80	0.75	0.78	0.82	0.77
Feed/kg gain ..kg	3.83	3.78	3.50	4.09	3.63	3.72	3.71	3.87	3.63	3.62
Overall Summary										
Daily feed ....kg	1.94	1.79	1.86	1.97	2.05	1.91	1.85	1.99	1.92	1.87
Daily gain ....kg	0.61	0.59	0.62	0.61	0.66	0.62	0.61	0.62	0.64	0.60
Feed/kg gain ..kg	3.18	3.03	3.00	3.23	3.11	3.08	3.04	3.22	3.00	3.11

[1] SBM = Soybean meal, RSM = Rapeseed meal

[2] This table does not include data from treatments 6, 7, 18 and 19 receiving iron and manganese supplements

Table 6: EXPERIMENT 1. EFFECTS OF DIETARY COPPER, IRON AND MANGANESE ON PERFORMANCE OF GROWING PIGS[1,2]

	Copper Levels (ppm)						
	0	125 to 40kg	125	200 to 40kg	200	0+Fe+Mn	200+Fe+Mn
Number of pigs	8	8	8	8	8	8	8
Av. initial weight ...kg	5.7	6.0	5.6	5.9	6.5	6.2	6.5
Av. final weight .....kg	77.5	75.8	76.6	77.2	76.5	77.7	76.9
Growth Data							
Initial - 40 kg							
Daily feed .....kg	1.15	1.12	1.27	1.14	1.28	1.24	1.25
Daily gain .....kg	0.46	0.49	0.48	0.48	0.48	0.47	0.51
Feed/kg gain ...kg	2.49	2.29	2.64	2.37	2.67	2.63	2.45
40 kg - 77 kg							
Daily feed .....kg	3.23	2.95	2.74	3.02	3.07	2.98	2.87
Daily gain .....kg	0.82	0.78	0.80	0.72	0.84	0.78	0.77
Feed/kg gain ...kg	3.95	3.78	3.42	4.19	3.66	3.83	3.73
Overall Summary							
Daily feed .....kg	2.00	1.79	1.94	1.97	2.07	1.96	1.97
Daily gain .....kg	0.62	0.59	0.64	0.60	0.65	0.61	0.63
Feed/kg gain ...kg	3.22	3.04	3.03	3.29	3.18	3.22	3.12

[1] To provide 41 ppm iron (Fe) as ferrous sulfate and 81 ppm manganese (Mn) as manganese sulfate.

[2] This table does not include data from treatments 8, 9, 10, 11 and 12 receiving the combination of soybean meal and rapeseed meal.

levels up to 250 ppm. The results in this experiment suggest that there is an advantage in leaving 200 ppm copper in the diet to market weight compared with removal at 40 kg liveweight. This observation is similar to that reported by Barber et al. (1957) but differs from the data presented by Lucas et al. (1961) suggesting that the overall effect of copper on rate of gain and efficiency of feed conversion to market weight is a reflection of its effect during the growing period.

Table 6 of Experiment 1 pools seven treatments over two protein sources to include treatments with added iron and manganese in addition to diets with or without dietary copper. Inclusion of iron and manganese in the diets of pigs did not affect feed intake, rate of gain or efficiency of feed conversion. In general, performance of pigs receiving iron and manganese without copper was similar to that of pigs receiving the control diet without copper, while the addition of iron and manganese to the copper-supplemented diet did not result in a more rapid gain than that of the pigs receiving 200 ppm copper. These results suggest that there is no advantage in feeding supplemental iron and manganese at the level employed in this experiment when diets are based on barley and wheat with SBM or RSM or both as protein supplements.

In Experiment 2 (Table 7), dietary copper did not significantly increase feed intake and efficiency of feed conversion. However, as in Experiment 1, pigs fed 200 ppm copper without zinc gained non-significantly more rapidly than pigs in any other treatments.

Table 7: EXPERIMENT 2. EFFECTS OF DIETARY COPPER, ZINC AND PROTEIN SOURCE ON PERFORMANCE OF GROWING GILTS AND BARROWS

	Copper and Zinc Levels (ppm)				Protein			Sex	
	Cu 0 Zn 0	125 0	200 0	200 200	SBM	SBM+RSM	RSM	M	F
Number of pigs	12	12	12	12	16	16	16	24	24
Av. initial weight ....kg	10.5	9.9	10.6	10.6	10.6	10.5	10.2	10.4	10.4
Av. final weight .....kg	76.8	76.7	77.1	77.0	76.7	77.1	76.8	77.0	76.8
Growth Data									
Initial - 40 kg									
Daily feed .....kg	1.65	1.45	1.76	1.55	1.53	1.70	1.55	1.61	1.60
Daily gain .....kg	0.48	0.47	0.53	0.44	0.51	0.47	0.45	0.49	0.47
Feed/kg gain ...kg	3.43	3.08	3.32	3.52	3.00	3.62	3.44	3.28	3.40
40 kg - 77 kg									
Daily feed .....kg	2.86	2.80	3.10	3.01	2.99	2.99	2.73	3.05	2.83
Daily gain .....kg	0.73	0.79	0.80	0.75	0.82	0.79	0.67	0.77	0.76
Feed/kg gain ...kg	3.92	3.54	3.87	4.01	3.65	3.79	4.08	3.96	3.72
Overall Summary									
Daily feed .....kg	2.25	2.09	2.41	2.26	2.23	2.34	2.11	2.28	2.21
Daily gain .....kg	0.61	0.63	0.67	0.60	0.67a	0.63a	0.56b	0.63	0.62
Feed/kg gain ...kg	3.68	3.31	3.60	3.77	3.33	3.71	3.76	3.62	3.56

a, b Values in each row with a common letter or no letter are not significantly different ($P < 0.10$)

Generally, dietary copper resulted in a slight but non-significant increase in efficiency of feed utilization. Average feed intake was 2.41 kg per day, average gain 0.67 kg per day and efficiency of feed utilization 3.60 kg feed/kg gain which were similar to performance obtained in Experiment 1. The performance of pigs receiving additional zinc with copper was similar to that of pigs receiving the diet without supplemental zinc. These results indicate that the addition of 200 ppm zinc to diets containing high levels of copper did not improve pig performance. Data presented by Kline et al. (1972) demonstrated that addition of 200 or 300 ppm dietary zinc to copper-supplemented diets resulted in depressed growth as compared with copper-supplemented diets supplemented with 100 ppm zinc. Two hundred ppm zinc was added to a diet containing 50 ppm zinc in the present experiment and it is possible that this level of 250 ppm zinc was excessive.

(b) Effects of Dietary Protein

The effects of source of supplemental protein on performance of growing pigs in Experimental 1 are shown in Table 5. Feeding SBM, a combination of SBM and low glucosinolate RSM or RSM at isonitrogenous, isocaloric levels did not result in any significant difference in daily feed intake (average 1.91, 1.85 and 1.99 kg per day), rate of gain (average 0.62, 0.61 and 0.62 kg per day), or efficiency of feed utilization (average 3.08, 3.04

and 3.22 kg feed/kg gain) respectively. These results suggest that a low glucosinolate RSM could replace SBM on an equivalent protein and energy basis. These results contrast somewhat with those of Bowland (1972). In his report, gilt performance but not barrow performance was depressed when low glucosinolate RSM replaced SBM, but in the present study both sexes performed similarly when either RSM or SBM or a combination of the two provided the supplemental protein.

In Experiment 2 (Table 7) daily feed intake was not affected by the different sources of supplemental protein. Pigs fed SBM appeared to perform better than pigs fed either RSM or a combination of SBM and RSM in terms of rate of gain and efficiency of feed utilization. The daily gain for SBM-fed pigs averaged 0.67 kg compared with the lower ($P < 0.10$) mean value of 0.56 kg for pigs fed RSM or the intermediate daily average of 0.63 kg for pigs fed the combination of the two protein supplements. Efficiency of feed conversion averaging 3.33 kg per kg gain for pigs fed SBM was numerically better than the mean value of 3.76 kg feed per kg gain for pigs fed RSM or 3.71 kg feed per kg gain for pigs receiving the combination of SBM and RSM. The RSM used in this experiment was different from the low glucosinolate RSM used in Experiment 1, and this difference could explain the trends toward differing comparative results in the two experiments. In previous studies with standard commercial RSM, Bowland (1971, 1972) observed a depression in gain of market pigs, when 5 or 10 percent RSM was fed.

(c) Effects of Sex

In both experiments (Tables 5 and 7), gilts and barrows did not differ significantly in daily feed intake, daily rate of gain and efficiency of feed conversion. However, in Experiment 1, barrows ate non-significantly more than gilts with an average of 2.05 kg feed/day for barrows and 1.80 kg feed/day for gilts. In Experiment 2, feed consumption averaged 2.28 kg/day for barrows and 2.21 kg/day for gilts. Higher consumption by barrows in Experiment 1 resulted in a slightly (non-significant) increased gain but lower efficiency of feed conversion, an observation similar to that reported by Young et al. (1968), Skitsko and Bowland (1970) and Pierce and Bowland (1972).

DIGESTIBILITY AND RETENTION

(a) Effect of Mineral Supplements

Means for digestible N (DN), N-retention (RN), digestible energy (DE) and metabolizable energy (ME) for Experiments 1 and 2 are presented in Tables 8 and 9 respectively. Dietary copper, iron and manganese or zinc did not significantly ($P < 0.05$) alter DN or RN in either experiment. However, in Experiment 2, copper supplementation resulted in a non-significant increase in DN. Copper-fed pigs averaged 84.5% DN as compared with the mean of 82.6% DN for basal pigs. DE or ME was not significantly altered by feeding dietary copper or iron and manganese in Experiment 1.

Table 8: EXPERIMENT 1. MEANS FOR DIGESTIBLE NITROGEN (DN), N-RETENTION (RN), DIGESTIBLE ENERGY (DE) AND METABOLIZABLE ENERGY (ME) FOR GROWING PIGS

		Mineral Supplement (ppm)					Protein Source			Sex	
	Cu Fe Mn	0 0 0	125 0 0	200 0 0	0 + +	200 + +	SBM	SBM+RSM	RSM	M	F
Number of pigs		6	12	12	6	6	14	14	14	21	21
Av. weight	kg	19.7	20.1	17.4	18.6	17.8	19.5	17.3	19.3	19.9	17.5
DN	%	85.6	86.1	85.4	83.8	85.4	86.9	85.2	84.5	85.4	85.4
RN/N intake	%	42.8	39.8	40.0	39.2	37.8	41.8b	49.1a	39.2b	42.9	37.0
RN/DN	%	49.7	46.8	46.2	46.7	45.0	47.4b	57.6a	46.8b	47.1	43.8
DE	%	85.1	84.8	84.2	83.6	84.4	86.2	85.3	83.1	84.9	84.0
ME	%	81.9	82.0	80.0	78.9	78.7	82.5	81.8	79.8	81.7	79.3
ME/DE	%	96.4	96.2	93.7	93.4	93.9	95.1	95.5	95.6	96.0	94.8
DN/kg feed	g	22.0	23.2	23.0	20.6	23.4	23.7	22.6	22.0	22.0	24.3
RN/kg feed	g	10.9	9.7	10.6	9.5	10.5	11.3b	13.0a	10.3b	10.5	10.6
DE/kg feed	kcal	3416	3362	3359	3328	3431	3457	3363	3355	3415	3359
ME/kg feed	kcal	3293	3233	3147	3107	3223	3287	3213	3208	3279	3184

a, b Values in each row with a common letter or no letter are not significantly different ($P < 0.05$)

Table 9: EXPERIMENT 2. MEANS FOR DIGESTIBLE NITROGEN (DN), N-RETENTION (RN), DIGESTIBLE ENERGY (DE) AND METABOLIZABLE ENERGY (ME) FOR GROWING PIGS

	Copper and Zinc Levels (ppm)				Protein Source			Sex	
Cu / Zn	0 / 0	125 / 0	200 / 0	200 / 200	SBM	SBM+RSM	RSM	M	F
Number of pigs	6	6	6	6	8	8	8	12	12
Av. weight .....kg	20.0	19.3	21.6	19.7	22.5	19.6	21.5	20.1	22.2
DN ..... %	82.6	84.8	85.6	83.0	81.8	84.4	85.8	84.3	83.7
RN/N intake ... %	49.0	44.6	53.3	52.8	49.8	52.8	47.1	49.8	50.0
RN/DN ..... %	59.4	52.6	62.1	63.5	60.9	62.3	54.9	59.1	59.6
DE ..... %	83.8a	85.7b	85.9b	83.8a	84.4a	85.1b	84.9b	84.8	84.8
ME ..... %	81.8a	83.7b	84.2b	82.2a	82.3	83.2	83.4	82.9	83.1
ME/DE ..... %	96.7a	96.9ab	97.5c	97.3bc	97.5	97.1	96.7	96.8	97.4
DN/kg feed .... g	19.9	20.4	20.6	20.0	19.6	20.5	20.6	20.3	20.1
RN/kg feed .... g	11.8	11.1	12.9	13.0	11.9	13.2	11.4	12.0	12.4
DE/kg feed .. kcal	3336a	3499b	3481b	3380a	3352a	3437b	3482b	3419	3429
ME/kg feed .. kcal	3249	3367	3314	3255	3253	3298	3336	3278	3314

a, b, c Values in each row with a common letter or no letter are not significantly different ($P < 0.05$)

In Experiment 2, DE and ME were increased ($P < 0.05$) by feeding either 125 ppm or 200 ppm supplementary copper. The proportion of total DE metabolized was significantly increased only when 200 ppm copper with or without dietary zinc was fed. It appears that the benefit obtained from supplementing pig diets with 200 ppm copper is due, at least in part, to the action of that quantity of copper in increasing digestibilities of the energy and protein components of the diet. Additional zinc seems to nullify the benefit of copper by decreasing digestibility of the energy fraction. This effect could be associated with a direct interaction between zinc and copper resulting in slight depression in growth when high levels of zinc are fed.

(b) Effects of Source of Dietary Protein

In Experiment 1 (Table 8), percent DN was not significantly affected by the source of supplemental protein. However, percent of RN per kg of feed and RN as a percentage of gross N-intake was higher ($P < 0.05$) for pigs fed a combination of low glucosinolate RSM and SBM diets as compared with pigs fed either SBM or RSM diets. The proportion of RN of DN averaged 57.6% for the SBM-RSM meal which was significantly ($P < 0.05$) higher than the means of 47.4% and 46.8% obtained for SBM and RSM diets respectively. In Experiment 2 (Table 9), where standard RSM was used, percent DN was non-significantly higher for pigs fed either SBM-RSM or RSM alone than for pigs fed SBM. On the other hand, RN and the proportion

of RN of the total N-intake were non-significantly depressed by the standard RSM. Similar to the observation in Experiment 1, the combination of SBM and RSM resulted in increased retention values for N above the values for either SBM or RSM diets. Manns and Bowland (1963) noted a trend toward reduced digestibility of energy and N when pigs were fed 100% RSM in substitution for SBM. The data obtained in this study suggest that a combination of SBM and RSM has a complementary effect as protein supplements insofar as RN is concerned.

In Experiment 1, DE and ME were not affected by the source of protein supplement. Similar findings have been reported by Bell (1965). In Experiment 2, the DE values were higher ($P < 0.05$) for pigs fed either RSM alone or a combination of SBM-RSM than for pigs fed the SBM diet. Results similar to these were obtained for rats by Bowland and Standish (1965). There were no significant differences in the ME values amongst the three protein sources. However, the proportion of DE metabolized was slightly higher for SBM-fed pigs than for SBM-RSM or RSM-fed pigs.

(c) Effects of Sex

Gilts and barrows did not differ at $P < 0.05$ in any of the digestibility and retention criteria studied. Results of Brooks (1967) and Bowland (1972) differ from those obtained in this study as these workers obtained sex differences. However, other results including those presented by Anderson and Bowland (1967), Skitsko

and Bowland (1970) and Pierce and Bowland (1972) agree with the results reported herein. There is no apparent explanation as to why barrows and gilts may differ in digestibility and retention in some studies and not in others.

CARCASS DATA

(a) Effects of Copper Supplementation

The mean values for carcass measurements for market pigs in Experiment 1 are presented in Tables 10 and 11 and in Experiment 2 in Table12. In both experiments, the dressing percentages were non-significantly increased in pigs fed copper supplemented diets. Other workers (Lucas and Calder, 1957; Barber et al., 1960; Allen et al., 1961; and DeGoey et al., 1971) have observed a general trend toward increased dressing percentage of pigs fed dietary copper. Castell and Bowland (1968a) reported an increase in dressing percentage from feeding supplemental copper in one experiment, but in the other experiment observed a non-significantly decreased dressing percentage by feeding supplementary copper.

In Experiment 1, backfat thickness was reduced by the feeding of copper. This decrease was significant ($P < 0.05$) for pigs fed either 125 ppm dietary copper to market weight or 200 ppm to 40 kg liveweight. The reduced backfat for pigs fed 125 ppm copper to 40 kg liveweight or 200 ppm throughout the experiment was not significantly different from that of the basal pigs. It appears that if rate of gain is improved by copper supplementation as

Table 10: EXPERIMENT 1. EFFECTS OF DIETARY COPPER AND PROTEIN SOURCE ON CARCASS CHARACTERISTICS OF MARKET PIGS[1]

	Copper Levels (ppm)					Protein Source			Sex	
	0	125 to 40kg	125	200 to 40kg	200	SBM	SBM+RSM	RSM	M	F
Number of pigs	12	12	12	12	12	20	20	20	30	30
Carcass weight ...kg	61.7	59.4	59.5	60.1	60.2	59.4	60.6	60.5	60.5	59.9
Dressing ..... %	76.9	77.5	77.7	78.9	78.6	78.1	78.5	78.8	78.2	78.7
Total backfat ....cm[2]	10.3c	9.6bc	9.3ab	8.6a	9.9bc	9.7	9.5	9.4	9.7	9.4
Loin area .....sq cm	25.8	26.3	26.7	26.4	26.8	26.1	26.3	26.8	26.8	26.0
Ham/carcass ..... %	27.0	26.9	27.0	27.7	26.8	27.0	27.0	27.1	27.3	26.9
Lean in ham face.. %	48.7bc	48.3bc	50.4ab	51.3a	47.3c	47.9a	50.2b	49.5b	49.4	49.0
Carcass length ...cm	73.2	73.5	74.1	74.6	73.2	73.6	74.0	73.6	73.7	73.7
ROP score	68.4a	68.7ab	69.2ab	71.7b	68.0ab	68.5	69.2	69.5	68.8	69.4
Grade index	98.6ab	97.5a	98.0a	101.0b	97.5a	97.8	98.7	99.2	98.3	98.7

[1] This table does not include data from treatments 6, 7, 18 and 19 receiving iron and manganese supplements.

[2] Sum of three measurements (shoulder, loin and backfat).

a, b Values in each row with a common letter or with no letter are not significantly different ($P < 0.05$).

Table 11. EXPERIMENT 1. EFFECTS OF SUPPLEMENTARY COPPER, IRON AND MANGANESE ON CARCASS CHARACTERISTICS OF MARKET PIGS[1]

	Copper Levels (ppm)						
	0	125-40kg	125	200-40kg	200	0+Fe+Mn	200+ Fe+Mn
Number of pigs	8	8	8	8	8	8	8
Carcass weight ...kg	59.8	59.2	58.6	59.8	60.4	59.5	58.4
Dressing ..... %	77.7	77.3	77.3	79.3	78.6	76.6	76.7
Total backfat ....cm	10.4	9.5	9.4	8.5	10.0	10.4	8.4
Loin area .....sq cm	26.3	26.7	26.2	25.1	28.1	24.1	26.8
Ham/carcass ..... %	27.1	26.7	26.9	27.8	27.0	27.0	27.3
Lean in ham face.. %	48.7	48.4	48.4	50.4	47.7	49.2	52.8
Carcass length ...cm	73.1	73.4	73.4	74.9	73.1	74.1	74.1
ROP score	68.4	69.2	68.7	70.6	68.1	67.4	70.6
Grade index	98.4	97.5	97.4	101.0	98.1	98.6	97.6

[1] This table does not include data from treatments 8, 9, 10, 11 and 12 receiving the combination of SBM and RSM protein supplements.

Table 12: EXPERIMENT 2. EFFECT OF DIETARY COPPER, ZINC AND PROTEIN SOURCE ON CARCASS CHARACTERISTICS OF MARKET PIGS

	Copper and Zinc Supplements (ppm)				Protein Source			Sex	
Cu Zn	0 0	125 0	200 0	200 200	SBM	SBM+RSM	RSM	M	F
Number of pigs	12	12	12	12	16	16	16	24	24
Carcass weight .....kg	59.3	59.6	61.0	59.8	59.7	59.8	60.1	59.6	60.1
Dressing ..... %	77.2	77.7	79.1	77.6	77.8	77.6	78.3	77.4	78.3
Total backfat ..... cm	9.7	9.5	9.8	9.9	9.6	9.7	9.9	9.9	9.5
Loin area ..... sq cm[1]	25.0	27.3	28.1	27.0	27.0	26.8	26.8	26.0	27.7
Ham/carcass ..... %	27.1	27.9	27.1	27.5	27.7	27.1	27.4	27.0	27.8*
Lean ham in face ... %	50.1	50.6	51.4	50.1	49.9	50.5	51.7	50.6	50.7
Carcass length .... cm	72.4	72.9	72.4	72.8	73.2	72.6	72.1	72.5	72.8
ROP score	69.6	69.7	69.3	68.1	69.6	68.9	69.0	68.5	69.8
Grade index	97.3	98.0	99.2	97.9	98.9	98.4	97.0	97.9	98.3

[1] Sum of three measurements (shoulder, loin and backfat).

* Significantly different at $P < 0.05$.

occurred when 200 ppm copper was fed, the advantage in decreased backfat thickness is reduced compared with that obtained by copper levels where there is no effect on gain. In general, the results on backfat thickness in Experiment 1 agree with the data presented by Barber et al. (1960a, 1961b), Wallace et al. (1966b, 1968), Bekaert et al. (1967), Castell and Bowland (1968a) and DeGoey et al. (1971). However, it is in contrast with the results of Allen et al. (1961) and Barber et al. (1961a) who reported that copper supplementation may actually increase backfat thickness of pigs. Results obtained in Experiment 2 of this study did not show any definite pattern as regards the effect of copper feeding on backfat thickness.

There was a general trend for copper supplements to produce leaner carcasses as indicated by measures associated with carcass leanness. Loin area was non-significantly increased by feeding supplementary copper in both experiments. A similar effect upon the eye muscle was reported by Braude et al. (1962), Lucas et al. (1962), Castell and Bowland (1968a) and DeGoey et al (1971). The proportion of lean in ham face was significantly ($P < 0.05$) increased by feeding either 125 ppm copper to market weight or 200 ppm copper to 40 kg liveweight. However, feeding 125 ppm copper did not result in a significant increase in this ratio in Experiment 2.

The ham as a percentage of carcass and the carcass length were not affected by dietary copper. Barber et al. (1960a), Barber et al.

(1961a) and Bekaert et al. (1967) observed a general tendency for pigs which received supplemental copper to have shorter carcass length. However, other results including those presented by Barber et al. (1961b), Wallace et al. (1966) and DeGoey et al. (1971) agree with the results reported herein.

Feeding pigs 200 ppm supplementary copper to 40 kg liveweight resulted in a significantly ($P < 0.05$) higher ROP score and grade index over the basal pigs. These advantages seemed to be lost when pigs were fed 200 ppm copper to market. The smaller reduction in backfat thickness for pigs fed 200 ppm copper was partly responsible for the lower ROP rating and grade index for these pigs.

Zinc or iron and manganese supplements had no effect on any of the carcass characteristics. Similar results were obtained by DeGoey et al. (1971).

(b) Effects of Dietary Protein

Source of supplemental protein had no influence ($P < 0.05$) on dressing percentage, backfat thickness, loin area, carcass length, ROP score or grade index in either experiment (Tables 10 and 11). However, in Experiment 1, feeding a combination of SBM and RSM or RSM alone resulted in a significantly ($P < 0.05$) higher proportion of lean in the ham face compared with feeding SBM as the sole supplementary protein. In the experiments of Manns and Bowland (1963), carcass measurements and carcass grades were not significantly influenced by the addition

of solvent-extracted RSM as a replacement for up to 100% of the SBM in the ration. Similarly, Bell (1956) observed little influence of myrosinase-free RSM on carcass quality. These earlier workers, however, did not observe the proportion of lean in ham face as one of the carcass characteristics of pigs fed RSM. Higher lean in ham face coupled with lesser backfat thickness may be indicative of leaner carcasses brought about by higher RN for pigs fed the combination of SBM and RSM diets.

(c) Effects of Sex

Gilts and barrows did not vary significantly in most of the carcass characteristics in this study (Tables 10 and 11). However, there was a consistent trend toward lesser backfat thickness for the gilts than barrows, an observation consistent with that of numerous researchers including Wagner et al. (1963) and Friend and MacIntyre (1970). In Experiment 2, percent ham of the carcass in gilts was significantly ($P < 0.05$) higher than that for barrows. This agrees with the previous reports of Skitsko and Bowland (1970) and Young et al. (1968)

TISSUE COPPER LEVELS

(a) Liver

Tissue copper levels (dry matter basis) for Experiment 1 are presented in Tables 13 and 14. Dietary copper increased ($P < 0.05$)

the levels of copper in the liver. Liver copper concentration for pigs receiving no supplemental copper averaged 17.8 ppm, while the liver copper stores for those receiving 125 ppm copper averaged 99 ppm which was significantly ($P < 0.05$) lower than the mean of 378.9 ppm for pigs fed 200 ppm dietary copper. Bass et al. (1956), Allen et al. (1958), Young et al. (1970) and Kline et al. (1972) have reported levels of 1260, 887.5, 252.7 and 114 ppm copper on a dry matter basis respectively in livers of pigs fed 250 ppm copper.

A non-significantly lower mean of 81.9 ppm for copper in the liver was obtained for pigs fed 125 ppm dietary copper to 40 kg liveweight compared with the mean of 99.0 ppm when the same amount of supplementary copper was fed to market weight. The liver copper stores of pigs fed 200 ppm dietary copper to 40 kg liveweight was significantly ($P < 0.05$) lower than those that received the same amount of dietary copper to market (262.3 ppm versus 378.9 ppm). This result agrees with the findings of Lucas and Calder (1957) that removal of copper from the diet well in advance of market weight lowers copper levels stored in the liver compared with animals fed copper to market weight. However, in the present study copper levels in the livers of animals where supplemental copper was removed at 40 kg liveweight were still much higher than the levels of the control animals receiving the basal diet.

Addition of a combination of iron and manganese to diets containing 200 ppm copper did not offer any significant protection

Table 13: EXPERIMENT 1. EFFECTS OF DIETARY COPPER ON TISSUE COPPER LEVELS[1,2]

	Copper Levels (ppm)				
	0	125-40kg	125	200-40kg	200
Number of pigs	12	12	12	12	12
Liver	18.7a	83.8b	100.1b	266.0c	385.3d
Kidney	11.7a	73.7b	84.3b	89.4c	105.2d
Muscle[3]	3.9	3.8	4.2	4.0	4.2
Fat[4]	1.2	1.2	1.1	1.2	1.3

[1] This table does not include data from treatments 6, 7, 18 and 19 receiving iron and manganese supplements.

[2] Dry matter basis

[3] Longissimus dorsi from 7th and 8th rib of the left side.

[4] Backfat.

a, b, c, d Values in each row with a common letter or no letter are not significantly different ($P < 0.05$)

Table 14: EXPERIMENT 1. EFFECTS OF DIETARY COPPER, IRON, MANGANESE, PROTEIN SOURCE AND SEX ON TISSUE COPPER LEVELS[1,2]

	Mineral Supplement (ppm)							Protein Source		Sex	
Cu	0	125-40kg	125	200-40kg	200	0	200				
Fe	0	0	0	0	0	41	41	SBM	RSM	M	F
Mn	0	0	0	0	0	81	81				
Number of pigs	8	8	8	8	8	8	8	28	28	28	28
Liver	17.8a	81.9b	99.0b	262.3c	378.9d	18.3a	358.4d	168.8	178.8	177.6	169.9
Kidney	12.5a	73.2b	85.1b	87.3c	104.2d	12.3a	102.4d	69.5	66.9	66.9	69.4
Muscle[3]	4.0	4.0	4.3	3.9	4.3	4.2	4.1	3.9	4.2	4.1	4.1
Fat[4]	1.2	1.2	1.2	1.1	1.4	1.2	1.3	1.1	1.3	1.3	1.2

[1] Dry matter basis.

[2] This table does not include data from treatments 8, 9, 10 and 11 receiving the combination of SBM and RSM protein supplements.

[3] Longissimus dorsi from 7th and 8th ribs of the left side.

[4] Backfat

a, b, c, d Values in each row with a common letter or with no letter are not significantly different ($P < 0.05$).

against accumulation of copper in the liver. Copper levels in the liver of pigs fed additional iron and manganese with copper averaged 358.4 ppm while those fed dietary copper without iron and manganese averaged 378.9 ppm. Suttle and Mills (1966) and Kline et al. (1972) have presented similar results, but Kainski et al. (1967) indicated that iron and manganese lowered copper concentration in the liver. However, Kainski and coworkers employed 573 mg iron/kg feed in their experiment as compared with the values of 153 ppm total iron used in this study, 144 ppm and 118 ppm supplemental iron used by Suttle and Mills in their two experiments or 50 ppm dietary iron employed by Kline and coworkers.

In Experiment 2 (Table 15), the liver copper concentrations on a dry matter basis of copper-fed pigs were higher ($P < 0.05$), approximately six-fold, for pigs supplemented with 125 ppm copper and approximately twelve-fold for pigs supplemented with 200 ppm copper as compared with livers from unsupplemented pigs. These results follow the same general pattern as in Experiment 1. Adding 200 ppm zinc to the diet already containing 50 ppm zinc in addition to 200 ppm copper tended to reduce the liver copper stores of pigs, but the difference was not significantly ($P < 0.05$) different from those receiving 200 ppm copper without dietary zinc (259.9 ppm versus 312.8 ppm). Barber et al. (1960) reduced liver copper concentration of pigs fed 250 ppm copper from 836 ppm to 332 ppm on a dry matter basis by feeding an additional 250 ppm zinc. Similarly, DeGoey et al. (1971) decreased copper stores

Table 15: EXPERIMENT 2. EFFECTS OF DIETARY COPPER, ZINC AND PROTEIN SOURCE ON TISSUE COPPER STORE OF PIGS[1]

	Mineral Supplement (ppm)				Protein Source			Sex	
Cu Zn	0 0	125 0	200 0	200 200	SBM	SBM+RSM	RSM	M	F
Number of pigs	12	12	12	12	16	16	16	24	24
Liver	26.3a	154.0b	312.8c	259.9c	190.3	183.8	190.7	186.0	190.7
Kidney	14.3a	52.1b	104.3c	91.2c	66.4	65.8	64.2	65.6	65.3
Muscle[2]	3.4	3.7	3.5	3.5	3.5	3.4	3.6	3.5	3.5
Fat[3]	1.3	1.2	1.3	1.2	1.3	1.2	1.3	1.3	1.3

[1] Dry matter basis.

[2] Longissimus dorsi from 7th and 8th ribs of the left side.

[3] Backfat

a, b, c, d Values in each row with a common letter or no letter are not significantly different ($P < 0.05$).

of pigs fed 250 ppm copper from 466 ppm to 175 ppm on a moisture--free basis by feeding an additional 100 ppm each of zinc and iron.

(b) Kidney

In Experiment 1, feeding either 125 ppm or 200 ppm dietary copper significantly ($P < 0.05$) increased kidney copper levels as compared with kidneys from unsupplemented pigs (Tables 13 and 14). Similarly, kidney copper stores of pigs fed 200 ppm copper were significantly ($P < 0.05$) higher than those fed 125 ppm copper. The kidney copper concentration was non-significantly lowered by terminating 125 ppm copper supplementation at 40 kg liveweight as compared with feeding the same copper level to market weight. The mean of 87.3 ppm kidney copper concentration for pigs fed 200 ppm copper to 40 kg liveweight was significantly ($P < 0.05$) lower than the mean of 104.2 ppm for pigs fed 200 ppm to market (Table 14).

In Experiment 2 (Table 15), there was a four-fold increase in kidney copper levels of pigs fed 125 ppm copper and eight-fold increase for those receiving 200 ppm copper as compared with kidneys from unsupplemented pigs. These results follow the same general pattern as those from Experiment 1 and of some other reports (Hanrahan and O'Grady, 1968; Castell and Bowland, 1968b). In the present study, inclusion of 200 ppm additional zinc in a diet supplemented with 200 ppm copper resulted in a significant ($P < 0.05$) decrease in kidney copper concentrations when compared with kidneys

from pigs receiving 200 ppm copper without zinc. This result suggests that when pigs are fed high copper levels, both liver and kidney can obtain some protection against high copper accumulation if high levels of zinc are fed.

(c) Muscle

In both experiments, there was no difference ($P < 0.05$) in muscle copper levels (dry matter basis) for copper-fed pigs and basal pigs. In Experiment 1 (Table 14), the average muscle copper concentration for pigs receiving copper at either 125 ppm or 200 ppm level, fed either to 40 kg liveweight or market weight was 4.1 ppm compared with the level of 4.0 ppm for the basal pigs. In Experiment 2 (Table 15), pigs receiving 200 ppm copper with or without zinc supplementation averaged 3.5 ppm copper in their muscle while a mean of 3.7 ppm was obtained for pigs fed 125 ppm dietary copper. Basal pigs averaged 3.4 ppm. Investigating the effect of feeding 250 ppm dietary copper on the copper concentration of longissimus dorsi muscle, Taylor and Thomke (1964) found no difference between samples from basal and copper-fed pigs. In contrast, Bunch et al. (1961) reported an increase on a dry matter basis from 5.0 ppm to 16.0 ppm in the copper concentration in the loin muscle when 250 ppm supplementary copper was fed. Barber et al. (1961) reported that the copper concentration of the psoas muscle (dry matter basis) increased from 3.0 ppm to 5.0 ppm when the diet was supplemented with 250 ppm of copper. Castell and Bowland (1968b) reported a non-

-significant decline in copper concentration of muscle from 4.8 ppm to 2.4 ppm on a dry matter basis by feeding 250 ppm supplementary copper to growing pigs.

(d) Fat

There was no significant difference between copper levels in the fat of copper-fed pigs and those fed basal diets. In Experiment 1, pigs receiving copper at either 200 ppm fed to 40 kg liveweight or market weight and pigs receiving basal diets averaged 1.2 ppm copper on a dry matter basis in their backfat. Similar results were obtained in Experiment 2, where basal pigs averaged 1.3 ppm while copper supplemented pigs averaged 1.2 ppm copper in backfat.

(e) Effects of Protein Source

SBM or RSM or a combination of the two protein sources did not seem to have any influence on the ability of liver, kidney, muscle or fat to store copper. Similar to this finding is the report of Suttle and Mills (1966) who did not observe any alteration in copper metabolism due to the use of white-fish meal, dry skim milk or soya-bean meal as the source of supplemental protein for pigs fed 250 ppm copper.

(f) Effects of Sex

There was no difference in the copper concentration of liver, kidney, muscle or fat of barrows and gilts. There are no published studies indicating whether or not gilts and barrows store copper differently.

FATTY ACID COMPOSITION

(a) Effects of Copper

To facilitate presentation of the data, the sum of the saturated fatty acids (SFA) (14:0 + 16:0 + 18:0) and the sum of the unsaturated fatty acids (UFA) (16:1 + 18:1 + 18:2 + 18:3 + 20:1) were obtained (Tables 16, 17 and 18). Neither linolenic (18:3) nor gadoleic acid (20:1) was detected in Experiment 2. Together, the sum of both the SFA and the UFA represented greater than 98 percent of the fatty acids.

In both experiments, there was a numerically greater percentage (non-significantly different) of UFA for pigs receiving the copper supplemented diet compared with those fed basal diets.

In Experiment 1 (Table 16), there was a significantly ($P < 0.05$) greater amount of 16:1 in depot fat when 125 ppm copper was fed to market weight or when 200 ppm copper was fed to either 40 kg liveweight or market weight compared with basal pigs. In Experiment 2 (Table 18), there was no difference in the level of 16:1 in the depot fat of pigs fed basal or copper-supplemented diets. It is not apparent why the pigs differed between experiments in relation to the influence of dietary copper on levels of 16:1 fatty acid in the fat. In both experiments, copper supplementation resulted in increased percentages of 18:1 in depot fat. In Experiment 1, the amounts increased non-significantly with increase in level of copper supplementation and with length of time for which copper was fed. In Experiment 2, copper supplementation resulted in a significant

Table 16: EXPERIMENT 1. PERCENT FATTY ACID COMPOSITION OF DEPOT FAT OF PIGS AS AFFECTED BY COPPER SUPPLEMENTATION, SOURCE OF DIETARY PROTEIN AND SEX

	Copper Levels (ppm)					Protein Source			Sex	
	0	125 to 40kg	125	200 to 40kg	200	SBM	SBM+RSM	RSM	M	F
Number of animals	12	12	12	12	12	20	20	20	30	30
Fatty Acids										
14:0	1.5	1.8	1.7	1.8	1.5	1.6	1.6	1.8	1.6	1.7
16:0	25.7	25.3	24.7	24.7	24.2	25.8	24.6	24.4	25.4*	24.4
16:1	2.8a	3.1a	3.6b	3.8b	3.8b	3.3	3.5	3.5	3.2	3.6*
18:0	13.8	12.6	12.4	11.8	10.9	12.9	11.9	12.1	13.0*	11.5
18:1	45.7	46.1	47.1	46.4	48.3	46.0	47.3	46.9	46.0	47.0
18:2	7.7	8.3	8.2	8.8	8.4	7.9	8.3	8.5	7.9	8.7*
18:3 + 20:1	1.7a	1.8a	1.3b	1.8a	1.8a	1.4a	1.7b	1.9c	1.7	1.6
Σ Saturated	40.6	39.7	38.8	38.3	36.7	40.3	37.9	38.2	39.9*	37.6
Σ Unsaturated	58.3	59.3	60.2	60.8	62.3	58.6	61.0	60.9	59.0	61.3*

a,b Values in each row with a common letter or with no letter are not significantly different ($P < 0.05$).

* Significantly different at $P < 0.05$.

Table 17: EXPERIMENT 1. PERCENT FATTY ACID COMPOSITION OF DEPOT FAT OF PIGS FED COPPER, IRON AND MANGANESE SUPPLEMENTED DIETS[1]

	Copper Levels (ppm)						
	0	125-40kg	125	200-40kg	200	0+Fe+Mn	200+ Fe+Mn
Number of pigs	8	8	8	8	8	8	8
Fatty acids							
14:0	1.4	1.8	1.8	1.7	1.6	1.7	1.6
16:0	25.5	26.0	24.7	24.8	24.5	24.7	24.3
16:1	2.8a	3.4ab	3.2ab	3.8bc	3.8bc	2.8a	4.4c
18:0	13.8a	13.9a	12.5ab	11.2b	10.9b	12.6ab	10.1b
18:1	45.9	44.0	47.2	46.9	48.1	47.5	48.0
18:2	7.7	8.3	8.3	8.8	8.1	8.0	8.9
18:3 + 20:1	1.7b	1.8b	1.2a	1.8b	1.8b	1.7b	1.8b
Σ Saturated	40.7	41.7	39.0	37.8	37.0	37.2	36.0
Σ Unsaturated	58.2	57.5	60.0	61.3	61.9	61.6	63.0

[1] This table does not include data from treatments 8, 9, 10, 11 and 12 receiving the combination of SBM + RSM protein supplements.

a, b, c Values in each row with a common letter or no letter are not significantly different ($P < 0.05$).

Table 18: EXPERIMENT 2. EFFECTS OF DIETARY COPPER AND ZINC, PROTEIN SOURCE AND SEX ON THE PERCENT FATTY ACID COMPOSITION OF PORCINE DEPOT FAT

	Copper and Zinc Levels (ppm)				Protein Source			Sex	
Cu	0	125	200	200					
Zn	0	0	0	200	SBM	SBM+RSM	RSM	M	F
Number of pigs	12	12	12	12	16	16	16	24	24
Fatty Acids									
14:0	1.9	1.8	1.7	1.7	1.8	1.8	1.8	1.8	1.8
16:0	26.9	25.8	26.2	26.1	26.2	26.3	26.4	26.6*	25.9
16:1	4.8	5.1	4.7	4.7	4.7	5.0	4.8	5.0*	4.5
18:0	10.6	10.3	10.7	10.5	10.8	10.5	10.3	10.4	10.7
18:1	49.2a	50.1b	50.3b	50.4b	49.8	49.7	50.3	49.7	50.3
18:2	6.6	6.9	6.4	6.6	6.7	6.7	6.5	6.5	6.8
Σ Saturated	39.4	37.9	38.6	38.3	38.8	38.6	38.5	38.8	38.4
Σ Unsaturated	60.6	62.1	61.4	61.7	61.2	61.4	61.6	61.2	61.6

a, b Values in each row with a common letter or with no letter are not significantly different ($P < 0.05$).

* Significantly different at $P < 0.05$

($P < 0.05$) increase of 18:1 in depot fat.

The percent composition of 18:2 was also increased by copper in Experiment 1, but not in Experiment 2. The proportions of 18:3 and 20:1 were not affected by dietary copper.

Iron and manganese supplements in Experiment 1 resulted in no change in fatty acid composition of depot fat compared with that of pigs receiving the same levels of dietary copper without iron or manganese supplements. Supplemental zinc in addition to copper did not influence depot fat composition in Experiment 2.

There are three possible means by which a depot fat can be softened: by an increase in the proportion of UFA present; by an increase in the proportion of short chain fatty acids present; or by changes in the structure of the component triglycerides. The results of these experiments indicate that copper supplementation of the diet softens the depot fat of pigs by increasing the proportion of UFA, notably 18:1 present therein. Previous experiments at The University of Alberta (Bowland and Castell, 1964; Elliot and Bowland, 1968, 1969, 1970; and Myres and Bowland, 1972) support the observation that copper supplementation softens depot fat in pigs and that the reason for the softening is associated with the proportions of UFA and SFA present in the fat.

Taylor and Thomke (1964) have suggested that high levels of dietary copper could: influence the absorption of dietary fat components; influence the mobilization from or deposition of fatty acids in the depot fat; or that copper stored in the liver might

interfere with endogenous fat metabolism. This study does not reveal the mode of action of copper in fat metabolism.

An increase in linoleic acid (18:2) in the depot fat of copper supplemented pigs was observed in Experiment 1. Elliot and Bowland (1970) and Myres and Bowland (1972) also observed a similar increase in 18:2. Linoleic acid is an essential fatty acid generally not considered to be synthesized by the pig and therefore required in the diet, although Babatunde et al. (1968) have indicated that there appeared to be a net synthesis of this acid. Increases in the proportion of 18:2 in the backfat of pigs would tend to indicate that the effect of copper on fat metabolism may be one of either promoting preferential deposition of unsaturated fatty acids in the depot fat or, conversely, preventing mobilization of unsaturated fatty acid from the depot fat once deposited, as suggested by Taylor and Thomke (1964). This study, however, does not reveal the specific effects of copper on backfat composition of pigs. In general, the results of this investigation agree very closely with those reported by Elliot and Bowland (1970) and Myres and Bowland (1972) for barley-fishmeal diets.

(b) Effects of Dietary Protein

The total UFA in the depot fat of pigs receiving the three different protein supplements did not differ significantly ($P < 0.05$). In Experiment 1 (Table 16), the percent composition of the individual UFA: 16:1, 18:1 and 18:2 increased slightly in RSM or RSM + SBM fed

pigs compared with those fed SBM. The increase in the proportion of 18:3 + 20:1 was significantly($P < 0.05$) different amongst the three protein sources: 1.4%, 1.7% and 1.9% for SBM, RSM + SBM and RSM, respectively. Experiment 2 (Table 18), did not show any specific trend in the proportions of individual UFA as regards the three protein sources. Experiment 1 of this study seems to indicate that RSM diets may result in softer depot fat than SBM diets when fed to pigs but this was not supported by Experiment 2. The different sources of RSM used in the two studies could be the explanation for these differences.

(c) Effects of Sex

In Experiment 1 (Table 16), the depot fat of barrows contained lesser ($P < 0.05$) amounts of UFA and greater ($P < 0.05$) amounts of SFA than did that of gilts. This result supports similar findings by Friend and Cunningham (1967) and Elliot and Bowland (1970). The individual SFA, notably 16:0 and 18:0, were significantly ($P < 0.05$) higher for barrows than gilts, while the individual UFA, 16:1 and 18:2, were correspondingly higher ($P < 0.05$) for gilts than barrows.

In Experiment 2 (Table 18), the percent composition of the depot fat of gilts contained non-significantly ($P < 0.05$) higher amounts of UFA. 16:0 was significantly ($P < 0.05$) higher for barrows, while 18:1 and 18:2 were non-significantly ($P < 0.05$) higher for gilts.

GENERAL DISCUSSION

The responses to supplemental copper for pigs obtained in these experiments were similar to those reported in the literature. Because of the variations encountered, the results can be discussed only as indicative of trends rather than presenting conclusive evidence for proposed metabolic roles of supplementary copper.

One of the predominant effects observed during the studies was an improvement in rate of gain obtained by feeding 200 ppm copper and the increased efficiency in feed utilization from the addition of copper. Several research workers have reported a similar effect, notably Lucas et al. (1961), Lucas et al. (1962) and Kline et al. (1972). The present studies indicate an advantage in leaving copper in the diet to market weight and in feeding 200 ppm copper as compared with a level of 125 ppm. This finding is consistent with the suggestion of Hawbaker et al. (1961) that the action of supplementary copper could be a result of its effect on gut flora. Based on the digestion studies in Experiment 2, dietary copper increased protein and energy utilization, a finding which is still consistent with the suggestions of Hawbaker and coworkers.

The apparent beneficial growth effects of copper were lost by feeding 250 ppm zinc with 200 ppm copper in comparison with 50 ppm zinc and 200 ppm copper. This could be due to an antagonism resulting from competition between the two minerals for site of absorption and action as previously suggested by Starcher (1969). The antagonism of zinc on copper is further demonstrated by the effects of zinc on liver and kidney copper concentrations. Whether such antagonism is enteric, systemic or both is not revealed by this study.

The carcass data indicate that a considerable improvement in carcass quality was brought about by copper supplementation. The consistent increase in area of loin may be related to increased NR by copper-fed pigs. Lesser backfat thickness may be due to the action of copper in facilitating the metabolism of mobilized fat thereby reducing the rate of re-deposition. This aspect of the work requires further study.

The lipid analyses show that dietary copper increases the percent composition of UFA. This observed change could result from the alteration of known metabolic pathways for the endogenous synthesis of fatty acids. The apparent increase of 18:2 resulting from dietary copper in the first experiment may be, in fact, an increase in the 18:2 Δ 6, 9 unsaturated fatty acid which animals can synthesize rather than an increase in the 18:2 Δ 9, 12 unsaturated 18 carbon acid considered essential. This was previously suggested by Elliot and Bowland (1970).

The use of RSM that contained normal glucosinolate levels as a protein source in pig diets (Experiment 2) caused a reasonably large depression in growth (significant at $P < 0.10$) which differed from the results obtained from feeding low glucosinolate RSM. Glucosinolates may be implicated in depressed protein utilization resulting in depressed growth. The glucosinolate content of RSM has been one of the factors limiting the most efficient utilization of RSM by livestock. Genotypes of rape have been found which contain very low levels of glucosinolate in the seed and emphasis is being given in plant breeding programs to develop these strains and varieties for commercial use. (Rapeseed Association of Canada, 1972).

The research reported herein has many practical implications. Most research, including this study, designed to evaluate copper as growth promotant, has indicated that copper supplementation of the diet improves rate of gain and efficiency of feed conversion and results in pigs going to market at a younger age and at lower cost per unit of gain. The economic advantage of this to the farmer is further enhanced by the improved carcass quality of such copper-fed pigs, which includes higher dressing percentage, lesser backfat and larger loin area.

High level copper feeding has not been accepted by the Health Protection Branch, Department of National Health and Welfare, Canada or the Food and Drug Administration in the U.S.A.; one of the reasons being that high levels of copper fed over a long period of time may alter the bacterial population in lagoons so that proper oxidation

does not take place. The fecal material when spread may also add rather large quantities of copper to the soil. Although these considerations are of potential concern, there appears to be no published data to support the possibility that dietary copper poses a problem in these areas. It is, however, an area that should be researched.

Koch et al. (1968), have suggested that since the composition of porcine depot fats can easily be altered by dietary means, they could serve as a potential source of unsaturated fat in the human diet for the reduction of serum cholesterol levels. Based on the results presented herein, copper supplementation of pig diets as well as the use of a low glucosinolate RSM as a protein supplement for growing pigs, could offer a partial alternative to the use of highly unsaturated vegetable fats in the human diet.

On the other hand, lipid composition may be an important factor in meat quality and in the keeping characteristics of meat. Fats containing relatively high proportions of unsaturated fatty acids are subject to oxidation, the end products of which result in undesirable odors and flavors in the product with which the fat is associated. Therefore, dietary treatments such as copper supplementation or the use of low glucosinolate RSM, which increase the proportion of unsaturated fatty acids present in the depot fat, could result in adverse effects on the storage life of pork.

High copper concentrations were obtained in the livers and kidneys of the copper-fed pigs. Sheep livers normally

contain copper levels as high as those obtained for the livers from copper-supplemented pigs. Wynne and McClymont (1955) and Kline et al. (1971) obtained 306 and 769 ppm copper on a dry matter basis respectively for livers of sheep fed diets unsupplemented with copper as compared with the value of 77 ppm copper obtained by Kline and coworkers for livers of pigs fed 200 ppm copper in the same study or the high of 378.9 ppm copper of dry tissue obtained for livers of pigs fed 200 ppm copper in studies reported herein. Moreover, it is unlikely that hog liver and kidney will constitute a sufficiently large proportion of human diets to markedly influence copper intake. The muscle and fat tissues, which form a major component of human diets, were not increased in copper level by supplemental dietary copper.

Further research is required before the practical effects of copper supplementation and/or the use of RSM in swine diets on carcass quality and fatty acid composition of porcine depot fat can be fully elucidated.

GENERAL SUMMARY

Responses of pigs to inclusion of copper sulfate to supply 125 or 200 ppm copper in the feed were studied under conditions involving withdrawal of copper at 40 kg liveweight or continuous copper supplementation to market weight, feeding additional dietary manganese and iron in one experiment and inclusion of 200 ppm dietary zinc in another experiment. In both experiments, diets were supplemented with either soybean meal (SBM), rapeseed meal (RSM) or a combination of SBM and RSM. In Experiment 1, low glucosinolate RSM from B. napus, cultivar Bronowski was used, while a RSM from B. campestris cultivar Span was used in the second experiment.

Data from these studies indicate that there is an advantage in feeding 200 ppm rather than 125 ppm dietary copper and in retaining the 200 ppm copper to market weight rather than terminating supplementation at 40 kg liveweight. There was no apparent benefit from additional supplemental iron or manganese (41 and 81 ppm respectively). All diets contained 37 ppm manganese and 112 ppm iron. Supplemental zinc at a level of 250 ppm did not prove beneficial in increasing rate of growth compared with diets containing

50 ppm zinc but did reduce the liver and kidney copper stores.

From results of digestibility trials during the rearing period it can be suggested that pigs fed 200 ppm supplementary copper were utilizing their diet more efficiently than any other treatment group.

Carcass data suggest that dressing percentage, area of loin and lean in the ham face were increased and backfat thickness was reduced by copper supplementation of the diet, resulting in an overall improvement in carcass quality.

Lipid analyses indicated that copper feeding resulted in a decreased percentage of saturated fatty acids (SFA) and an increased percentage of unsaturated fatty acids (UFA) in the depot fat. The decrease in the proportion of SFA resulted from decrease in weight percent of 16:0 present in the depot fat, while increases in weight percent of 18:1 and 18:2 in the depot fats accounted for the increase in the proportion of UFA.

Comparison between the protein sources indicated that a low glucosinolate RSM could replace SBM on an equivalent protein and energy basis. The standard RSM depressed growth and was inferior to SBM as a protein supplement for pig diets. Carcass quality was not significantly different when either SBM or RSM were fed, although RSM apparently improved carcass quality, increased the percent composition of UFA and decreased the percent composition of SFA in backfat.

BIBLIOGRAPHY

Allen, M.M., R.S. Barber, R. Braude and K.G. Mitchell. 1958. Copper and zinc supplements for fattening pigs. Proc. Nutr. Soc. 17: xii.

Allen, M.M., R.S. Barber, R. Braude and K.G. Mitchell. 1961. Further studies on various aspects of the use of high-copper supplements for growing pigs. Brit. J. Nutr. 15: 507-522.

Anderson, J. 1972. Wet digestion versus dry ashing for the analysis of fish tissue for trace minerals. Atomic Absorption Newsletter 11: 88-89.

Association of Official Agricultural Chemists. 1960. Official methods of analysis. 9th ed., Washington, D.C.

Association of Official Agricultural Chemists. 1965. Official methods of analysis. 10th ed., Washington, D.C.

Babatunde, G.M., W.G. Pond, E.F. Walker, Jr. and P. Chapman. 1968. Effect of dietary safflower oil or hydrogenated coconut oil on heart, liver and adipose tissue fatty acid levels and physical carcass measurements of pigs fed a fat-free diet. J. Animal. Sci. 27: 1290-1295.

Barber, R.S., J.P. Bowland, R. Braude, K.G. Mitchell and J.W.G. Porter. 1961. Copper sulphate and copper sulphide (CuS) as supplements for growing pigs. Brit. J. Nutr. 15: 189-197.

Barber, R.S., R. Braude and K.G. Mitchell. 1955a. Effect of adding copper to the diet of suckling pigs on creep meal consumption and liveweight gain. Chem. and Ind. 33: 1554.

Barber, R.S., R. Braude and K.G. Mitchell. 1955b. Antibiotic and copper supplements for fattening pigs. Brit. J. Nutr. 9: 378-381.

Barber, R.S., R. Braude and K.G. Mitchell. 1960. Further studies on antibiotic, copper and zinc supplements for growing pigs. Brit. J. Nutr. 14: 499-508.

Barber, R.S., R. Braude, K.G. Mitchell and J. Cassidy. 1955. High copper mineral mixture for fattening pigs. Chem. and Ind. 33: 601.

Barber, R.S., R. Braude, K.G. Mitchell, J.A.F. Rook and J.G. Rowell. 1957. Further studies on antibiotic and copper supplements for fattening pigs. Brit. J. Nutr. 11: 70-79.

Barber, R.S., R. Braude, K.G. Mitchell and J.W.G. Porter. 1960. Copper sulphate and copper sulphide (CuS) as supplements for growing pigs. Proc. Nutr. Soc. 19: xxvi-xxvii.

Barber, R.S., R. Braude, A.G. Chamberlain and K.G. Mitchell. 1961. Effect of feeding copper sulphate to bacon pigs for different intervals during the growing period. VIIIth Int. Congr. Animal Prod., Hamburg 3: 86-95.

Bass, B., J.T. McCall, H.D. Wallace, G.E. Combs, Jr., A.Z. Palmer and J.E. Carpenter. 1956. High level copper feeding for growing-fattening swine. J. Animal Sci. 15: 1230 (Abst.).

Bayley, H.S., C.Y. Cho and J.D. Summers. 1969. Growth and digestibility studies to evaluate rapeseed meal as a protein supplement for swine. Can. J. Animal Sci. 49: 367-373.

Beames, R.M. and L.E. Lloyd. 1964. Influence of soybean meal upon the response to copper. J. Animal Sci. 23: 1206 (Abst.).

Bearn, A.G. and H.G. Kunkel. 1954. Localization of Cu^{64} in serum fractions following oral administration: An alteration in Wilson's disease. Proc. Soc. Exp. Biol. Med. 85: 44-48.

Bekaert, H., W. Eeckhout and F. Buysee. 1967. L'influence de $CuSO_4$ et de CuO, ainsi que du degré granulometrique de $CuSO_4$ et d'un supplément de zinc sur les résultats d'engraissement et la teneur en cuivre de foie chez des porcs à léngrais. Revue de l'Agriculture, Nos. 11-12: 1571-1586.

Bell, J.M. 1965. Growth depressing factors in rapeseed meal. VI. Feeding value for growing-finishing swine of myrosinase-free, solvent extracted meal. J. Animal Sci. 24: 1147-1151.

Bell, J.M. and T.J. Belzile. 1965. Goitrogenic properties. Pp. 45-60 in Rapeseed meal for livestock and poultry - a review. Can. Dept. Agr. Publ. 1257. Ottawa, Canada.

Bellis, D.B. 1961. Supplementation of bacon pig rations by auremycin and two levels of copper sulphate. Anim. Prod. 3: 89-95.

Bendall, J.R. 1964. Meat proteins in Symposium on Foods; Proteins and their reactions. Pp. 225-254. H.W. Schultz and A.F. Anglemier, eds. The A.V.I. Publ. Co., Westport, Conn.

Berek, G., L. Urbanyi and T. Lakatos. 1967. A note on copper sulphate in the diet of pigs. Anim. Prod. 9: 421-424.

Bowland, J.P. 1954. Supplemental minerals for pigs. 33rd Annual Feeders' Day Report, Dept. Animal Sci., University of Alberta, Edmonton, Alberta, pp. 10-12.

Bowland, J.P. 1963. Antibiotic and copper sulfate supplements for growing pigs. 42nd Annual Feeders' Day Report, Dept. Animal Sci., University of Alberta, Edmonton, Alberta, pp. 12-14.

Bowland, J.P. 1966. Feeding value of rapeseed meal for swine. Pp. 69-80 in Rapeseed meal for livestock and poultry - a review. Can. Dept. Agr. Publ. 1257, Ottawa, Canada.

Bowland, J.P. 1971. Rapeseed as an energy and protein source in diets for growing pigs. Can. J. Animal Sci. 51: 503-510.

Bowland, J.P. 1972. Unprocessed rapeseed treated with propionic acid in diets of growing pigs: Performance, energy and protein digestibility and nitrogen retention, carcass measurement and fatty acid composition of backfat. Can. J. Animal Sci. 52: 553-562.

Bowland, J.P. and R.T. Berg. 1959. Influence of strain and sex on the relationship of protein to energy in the rations of growing and finishing bacon pigs. Can. J. Animal Sci. 39: 102-114.

Bowland, J.P., R. Braude, A.G. Chamberlain, R.F. Clascock and K.G. Mitchell. 1961. The absorption, distribution and excretion of labelled copper in young pigs given different quantities, as sulphate or sulphide, orally or intravenously. Brit. J. Nutr. 15: 59-72.

Bowland, J.P. and A.G. Castell. 1965. Supplemental copper for market pigs. 44th Annual Feeders' Day Report, Dept. Animal Sci., University of Alberta, Edmonton, Alberta, p. 5.

Bowler, R.J., R. Braude, R.C. Campbell, J.N. Craddock-Turnbull, H.F. Fieldsend, E.K. Griffiths, I.A.M. Lucas, K.G. Mitchell,

N.J.D. Nickalls and J.H. Taylor. 1955. High-copper mineral mixture for fattening pigs. Brit. J. Nutr. 9: 358-362.

Braude, R. 1945. Some observations on the need for copper in the diet of fattening pigs. J. Agric. Sci. 35: 163-167.

Braude, R. 1948. Some observations on the behaviour of pigs in an experimental piggery. Bull. Animal Behaviour 1 (6): 17-25.

Braude, R., M.J. Townsend, G. Harrington and J.G. Rowell. 1962. Effects of oxytetracycline and copper sulphate, separately and together, in rations for growing pigs. J. Agric. Sci. 58: 251-256.

Braude, R. 1965. Copper as a growth stimulant in pigs (cuprum pro pecunia). In cuprum provita (Transactions of a Symposium). Copper Development Assoc., London, pp. 55-66.

Braude, R. 1967. Copper as a stimulant in pig feeding. World Rev. Animal Prod. 3: 69-81.

Braude, R., K.G. Mitchell, M.J. Newport and R.J. Pittman. 1970. Response to different levels of supplementation with copper sulphate of diets for growing pigs. Proc. Nutr. Soc. 29: 10A.

Brooks, C.C. 1967. Effect of sex, fat, fiber, molasses and thryoprotein on digestibility of nutrients and performance of growing swine. J. Animal Sci. 26: 495-499.

Bunch, R.J., V.C. Speer and V.W. Hays. 1960. Effects of copper oxide, copper sulfate and chlortetracycline on performance of baby pigs. J. Animal Sci. 19: 1252 (Abst.).

Bunch, R.J., V.C. Speer, V.W. Hays, J.H. Hawbaker and D.V. Catron. 1961. Effects of copper sulfate, copper oxide and chlortetracycline on baby pig performance. J. Animal Sci. 20: 723-726.

Bush, J.A., J.P. Mahoney, H. Markowitz, C.J. Gubler, G.E. Cartwright and M.M. Wintrobe. 1955. Studies on copper metabolism. XVI. Radioactive copper studies in normal subjects and patients with hepatolenticular degeneration. J. Clin. Invest. 34: 1766-1778.

Cahilly, G.M. Jr., R.F. Miller, R.F. Kelly and C.C. Brooks. 1963. Effect of various levels of dietary lysine on certain blood phenomena, muscle development and muscle-protein biological value of growing swine. J. Animal Sci. 22: 726-732.

Carnes, W.H., G.S. Shields, G.E. Cartwright and M.M. Wintrobe. 1970. Vascular lesions in copper deficient swine. Fed. Proc. 20: 118 (Abst.)

Carrico, R.J. and H.F. Deutsch. 1969. Isolation of human hepatocuprein and cerebrocuprein: Their identity with erythrocuprein. J. Biol. Chem. 244: 6087-6093.

Carrico, R.J. and H.F. Duetsch. 1970. The presence of zinc in human cytocuprein and some properties of the apoprotein. J. Biol. Chem. 245: 723-727.

Cartwright, G.E. and M.M. Wintrobe. 1964. Copper metabolism in normal subjects. Am. J. Clin. Nutr. 14: 224-232.

Cassidy, J. and J.K. Eva. 1958. Relationship between the copper and iron concentrations in pigs' livers. Proc. Nutr. Soc. 17: xxxi.

Castell, A.G. and J.P. Bowland. 1968. Supplemental copper for swine: Growth, digestibility and carcass measurements. Can. J. Animal Sci. 48: 403-413.

Castell, A.G. and J.P. Bowland. 1968. Supplemental copper for swinw: Effects upon hemoglobin, serum proteins and tissue copper levels. Can. J. Animal Sci. 48: 415-424.

Chase, M.S., C.J. Gubler, G.E. Cartwright and M.M. WIntrobe. 1952. Studies on copper metabolism. IV. The influence of copper on the absorption of iron. J. Biol. Chem. 199: 757-764.

Chou, W.S., J.E. Savage and B.L. O'Dell. 1968. Relation of monoamine oxidase activity and collagen crosslinking in copper deficient and control tissues. Proc. 1st Intern. Symp. Trace Elem. Metab. Anim. 362-366. Ed. C.F. Mills (London).

Christie, W.W. and J.H. Moore. 1969. The effect of dietary copper on the structure and physical properties of ad'pose tissue triglycerides in pigs. Lipids 4: 345-349.

Comar, C.L., L. Singer and G.K. Davis. 1949. Molybdenum metabolism and interrelationships with copper phosphorus. J. Biol. Chem. 180: 913-922.

Combs, G.E., C.B. Ammerman, R.L. Shirley and H.D. Wallace. 1966. Effect of source and level of dietary protein on pigs fed high-copper rations. J. Animal Sci. 25: 613-616.

Cox, D.H. and O.M. Hale. 1962. Liver iron depletion without copper loss and swine fed excess zinc. J. Nutr. 77: 225-228.

Crampton, R.F., D.M. Matthews and R. Poisner. 1964. The absorption of copper by sacs of hamster small intestine. J. Physiol. 170: 27P-28P.

DeGoey, L.W., R.C. Wahlstsom and R.J. Emerick. 1971. Studies of high level copper supplementation to rations for growing pig. J. Animal Sci. 33: 52-57.

Dick, A.T. 1953. The control of copper storage in the liver of sheep by inorganic sulphate and molybdenum. Australian Vet. J. 29: 233-239.

Dick, A.T. 1954. Preliminary observations on the effect of high intakes of molybdenum and of inorganic sulphate on blood copper and on fleece character in crossbred sheep. Australian Vet. J. 30: 196-201.

Dick, A.T. 1955. Molybdenum in animal nutrition. Soil Sci. 81: 229-236.

Dowdy, R.P. and G. Matrone. 1968. A copper-molybdenum complex: Its effect and movement in the piglet and sheep. J. Nutr. 95: 197-201.

Dowdy, R.P. and G. Matrone. 1968. Copper-molybdenum in sheep and chicks. J. Nutr. 95: 191-196.

Dowdy, R.P. 1969. Copper metabolism. Am. J. Clin. Nutr. 22: 887-892.

Drouliscos, N.J., J.P. Bowland and J.I. Elliot. 1969. Influence of supplemental dietary copper on copper concentration of pig blood selected tissues and digestive tract contents. Can. J. Animal Sci. 50: 113-120.

Duncan, G.D., L.F. Gray and L.J. Daniel. 1953. Effect of zinc on cytochrome oxidase activity. Proc. Soc. Exp. Biol. Med. 83: 625-627.

Elliot, J.I. and Bowland, J.P. 1968. Effects of dietary copper sulphate on the fatty acid composition of porcine depot fats. J. Animal. Sci. 27: 956-960.

Elliot, J.I. and Bowland, J.P. 1969. Correlation of melting point with the sum of the unsaturated fatty acids in samples of porcine depot fat. Can. J. Animal Sci. 49: 397-398.

Elliot, J.I. and J.P. Bowland. 1970. Effects of dietary copper sulphate and protein on the fatty acid composition of porcine fat. J. Animal Sci. 30: 923-930.

Evans, G.W. and R.W. Cornatzer. 1970 Copper and zinc metalloprotenis in the rat. Fed. Proc. 20: 695 (Abst.).

Evans, G.W., P.F. Majors and W.E. Cornatzer. 1970. Mechanism for cadmium and zinc antagonism of copper metabolism. Biochem. Biophys. Res. Comm. 40: 1142-1148.

Evans, G.W., P.F. Majors and W.E. Cornatzer. 1970. Induction of cerulopasmin synthesis by copper. Biochem. Biophys. Res. Comm. 41: 1120-1125.

Evvard, J.M. , V.E. Nelson and W.E. Sewell. 1928. Copper salts in nutrition. Iowa Acad. Sci. 35: 211.

Folch, J., M. Lees and G.H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

Follis, R.H., Jr., J.A. Buch, G.E. Cartwright and M.A. Wintrobe. 1955. Studies on copper metabolism. John Hopkins Hosp Buld. 97, 405.

Friend, D.W. and H.M. Cunningham, 1967. Growth, carcass, blood and fat studies with pigs fed once or five times daily. J. Animal Sci. 26: 316-322.

Friend, D.W., J.M. MacIntyre. 1970. Paired feeding and metabolism trials comparing barrows with gilts. J. Animal Sci. 30: 931-934.

Gallagher, C.H., J.D. Judah and K.R. Rees. 1956. The biochemistry of copper deficiency. II. Synthetic process. Proc. Roy. Soc. 145: 195-205.

Gipp, W.F., W.G. Pond and S.E. Smith. 1967. Effects of level of dietary copper, molybdenum, sulfate and zinc on body weight gain, hemoglobin and liver copper storage for growing pigs. J. Animal Sci. 26: 727-730.

Gitlin, D., W.L. Hughes and C.A. Janeway. 1960. Absorption and excretion of copper in mice. Nature. 188: 150-151.

Gray, L.F. and G.H. Ellis. 1949. Some interrelationships of copper, molybdenum, zinc and lead in nutrition of the rat. J. Nutr. 40: 441-452.

Greer, M.A. 1956. Isolation from rutabaga seed of progoitrin, the precursor of the naturally occurring antithyroid compound, goitrin (L-5-vinyl-2-thiooxazolidone). J. Am. Chem. Soc. 78: 1260-1261.

Gubler, C.J., M.E. Lahey, G.E. Cartwright and M.M. Wintrobe. 1953. Studies on copper metabolism. IX. The transportation of copper in blood. J. Clin. Invest. 32: 405-414.

Gubler, C.J., G.E. Cartwright and M.M. Wintrobe. 1957. Studies on copper metabolism. XX. Enzyme activities and iron deficiencies. J. Biol. Chem. 224: 533-546.

Hanrahan, T.J. and J.F. O'Grady. 1968. Copper supplementation of pig diets. The effect of protein level and zinc supplementation on the response to added copper. Anim. Prod. 10: 423-432.

Harris, D.I.M. and A. Sas-Kortsak. 1967. The influence of amino acids on copper uptake by rat liver slices. J. Clin. Invest. 46: 659-667.

Hart, E.B., H. Steenbock, J. Waddell and C.A. Elvehjem. 1928. Iron in nutrition. IV. Copper as a supplement to iron for hemoglobin building in the rat. J. Biol. Chem. 77: 797-812.

Hartmans, J. and M.S.M. Bosman. 1970. Differences in the copper status of grazing and housed cattle and their biochemical background. Proc. 1st Intern. Symp. Trace Elem. Metab. Anim. 362-366. Ed. C.F. Mills (London).

Hawbaker, J.A., V.C. Speer, V.W. Hays and D.V. Catron. 1961. Effect of copper sulfate and other chemotherapeutics in growing swine rations. J. Animal Sci. 20: 163-167.

Hazelrig, J.B. and C.A. Owen. 1966. A mathematical model for copper metabolism and its relation to Wilson's disease. Am. J. Physiol. 211: 1075-1081.

Hill, C.H. and G. Matrone. 1962. A study of copper and zinc interrelationships. Proc. 12th World Poultry Congress., pp. 219-223.

Hill, C.H., G. Matrone, W.L. Payne and C.W. Barber. 1963. In vivo interactions of cadmium with copper, zinc and iron. J. Nutr. 80: 227-235.

Hill, C.H., B. Starcher and G. Matrone. 1964. Mercury and Silver interrelationships with copper. J. Nutr. 83: 107-110.

Hill, C.H. 1969. A role of copper in elastin formation. Nutr. Rev. 27: 99-100.

Holmberg, C.G. and C.B. Laurell. 1948. Investigation in serum copper. II. Isolation of the copper containing protein and a description of some of its properties. Acta Chem. Scan. 2: 550-556.

Hussar, N. 1958. Rapeseed oil meal studies with swine and rats. M.Sc. thesis, University of Alberta, Edmonton, Canada.

Kagi, J.H.R. and B.L. Vallee. 1960. Methallothionein, a cadmium and zinc-containing protein from equine renal cortex. J. Biol. Chem. 235: 3460-3465.

Kagi, J.H.R. and B.L. Vallee. 1961. Metallothionein, a cadmium and zinc-containing protein from equine renal cortex. II. Physicochemical properties. J. Biol. Chem. 236: 2435-2442.

Kainski, M.H., M.E. Zinn, R.A. Merkel and J.H. Hall. 1967. Effect of iron and copper intake on iron, copper and myoglobin levels in selected pig tissues. J. Agric. Ed. Chem. 15: 721-725.

King, J.O.L. 1964. The effect of the level of protein in the diet on the efficiency of copper sulphate as a growth stimulant for pigs. Brit. Vet. J. 120: 531-534.

Kinnamon, K.E. 1966. Copper, molybdenum and zinc interrelationships: The influence of inorganic sulfate upon distribution and excretion of ^{65}Zn and ^{99}Mo in pregnant rats. J. Nutr. 89: 365-372.

Kirchgessner, M. and E. Grassman. 1970. The dynamics of copper absorption. Proc. 1st Intern. Symp. Trace Elem. Metab. Anim. 277-298. Ed. C.F. Mills (London).

Kirchgessner, M. and U. Wesser. 1965. Copper absorption. Z. Tierphysiol. Tierernachr. Futtermittelk. 20: 44.

Kjaer, A. 1960. The chemistry of organic natural products, p. 122. Springer-Verlag, Vienna.

Kline, R.D., V.W. Hays and G.L. Cromwell. 1971. Effects of copper, molybdenum and sulfate on performance, hemotology and copper stores of pigs and lambs. J. Animal Sci. 33: 771-779.

Kline, R.D., V.W. Hays and G.L. Cromwell. 1972. Related effects of copper, zinc and iron on performance, hematology and copper stores of pigs. J. Animal Sci. 34: 393-396.

Koch, D.E., A.M. Pearson, W.T. Magee, J.A. Hoefer and B.S. Schweigert. 1968. Effect of diet on the fatty acid composition of pork fat. J. Animal Sci. 27: 360-365.

Kulwich, R., S.L. Hansard, C.L. Comar and G.K. Davis. 1953. Copper, molybdenum and zinc interrelationships in rat and swine. Proc. Soc. Exp. Biol. Med. 84: 487-491.

Lahey, M.E., C.J. Gubler, M.S. Chase, G.E. Cartwright and M.M. Wintrobe. 1952. Studies on copper metabolism. II. Hematologic manifestations of copper deficiency in swine. Blood 7: 1053-1074.

Lee, G.R., G.E. Cartwright and M.M. Wintrobe. 1968. Heme biosynthesis in copper deficiency in swine. Proc. Soc. Exp. Biol. Med. 127: 977-981.

Livingstone, R.M. and D.M.S. Livingston. 1968. Copper sulphate and antibiotics as feed additives for early weaned pigs. J. Agric. Sci. 71: 419-424.

Lodhi, G.N., D.R. Clandinin and R. Renner. 1970. Factors affecting the metabolizable energy value of rapeseed meal. I. Goitrogens. Poultry Sci. 49: 289-294.

Lucas, I.A.M. and A.F.C. Calder. 1957. Antibiotics and a high level of copper sulphate in rations for growing bacon pigs. J. Agric. Sci. 54: 81-99.

Lucas, I.A.M., R.M. Livingstone and I. McDonald. 1961. Copper sulphate as a growth stimulant for pigs. Effect of level and purity. Anim. Prod. 3: 111-119.

Lucas, I.A.M., R.M. Livingstone and A.W. Boyne. 1962. Copper sulphate as a growth stimulant for pigs. Effect of composition of diet and level of protein. Anim. Prod. 4: 177-183.

Lucas, I.A.M. 1964. Modern methods of pig nutrition: The impact of recent research. Vet. Rec. 76: 101-109.

Magee, A.C. and G. Matrone. 1960. Studies on growth, copper metabolism and iron metabolism of rats fed high levels of zinc. J. Nutr. 72: 233-242.

Mahoney, J.P., J.A. Bush, C.J. Gubler, W.H. Moretz, G.E. Cartwright and M.M. Wintrobe. 1955. Studies on copper metabolism. XV. The excretion of copper by animals. J. Lab. Clin. Med. 46: 702-708.

Manns, J.G. and Bowland, J.P. 1963. Solvent-extracted rapeseed oil meal as a protein source for pigs and rats. II. Nitrogen digestibility and retention; and energy and dry matter digestibility. Can. J. Animal Sci. 43: 264-270.

Matrone, G. 1960. Interrelationships of iron and copper in the nutrition and metabolism of animals. Fed. Proc. 19: 659-665.

Martson, H.R. and S.H. Allen. 1967. Function of copper in the metabolism of iron. Nature 215: 645-646.

Middleton, G. and Stuckey, R.E. 1953. The preparation of biological material for the determination of trace metals. Analyst. 78: 532-542.

Miller, R.F. and R.W. Engel. 1960. Interrelationships of copper, molybdenum and sulfate sulfur in nutrition. Fed. Proc. 19: 666-671.

Miller, E.J., G.R. Martin, C.E. Mecca and K.A. Piez. 1965. The biosynthesis of elastin cross-links: The effect of copper deficiency and a lathyrogen. J. Biol. Chem. 240: 3623-3627.

Mills, C.F. 1955. Availability of copper in freeze-dried herbage extracts to copper-deficient rats. Brit. J. Nutr. 9: 398-409.

Mitchell, K.G. 1953. Observations on the effect of adding copper to the diet of suckling piglets. Chem. and Ind. 31: 871-872.

Moore, J.W., W.W. Christie, R. Braude and K.G. Mitchell. 1968. The effect of 250 ppm of copper in the diet of growing pigs on the fatty acid composition of the adipose tissue lipids. Proc. Nutr. Soc. 27: 45A-46A.

Morrison, W.R. and L.M. Smith. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5: 600-608.

Myres, A.W., T.A. Omole and J.P. Bowland. 1972. Influence of high level copper supplements in diets for growing pigs. 51st Annual Feeders' Day Report. Dept. Animal Sci., University of Alberta, Edmonton, Alberta, pp. 9-13.

Myres, A.W. and J.P. Bowland. 1972. Effects of high levels of dietary copper on endogenous lipid metabolism in the pig. Can. J. Animal Sci. 52: 113-123.

National Academy of Sciences - National Research Council. 1968. Nutrient requirements for domestic animals. No. 2. Nutrient requirements of swine. Sixth revised edition. Publ. 1599; Washington, D.C.

Neumann, P.Z. and A. Sass-Kortsak. 1967. The state of copper in human serum; Evidence for an amino acid bound fraction. J. Clin. Invest. 46: 646-658.

O'Dell, B.L., B.C. Hardwick and G. Reynolds. 1961. Mineral deficiencies of milk and congenital malformations in rats. J. Nutr. 73: 151-157.

O'Dell, B.L., D.W. Bird, D.L. Ruggles and J.E. Savage. 1966. Composition of aortic tissue from copper-deficient chicks. J. Nutr. 88: 9-14.

O'Donovan, P.B., T.A. Spillance and J.F. O'Grady. 1966. A note on copper retention by pigs fed on various protein sources. Anim. Prod. 8: 333-336.

O'Hara, P.J., A.P. Newmand and R. Jackson. 1960. Parakeratosis and copper poisoning in pigs fed a copper supplement. Australian Vet. J. 366: 225-231.

Owen, C.A. and J.B. Hazelrig. 1966. Metabolism of Cu^{64}-labelled copper by the isolated raw liver. Am. J. Physiol. 210: 1059-1064.

Pulido, P., J.H.R. Kagi and B.L. Vallee. 1966. Isolation of some properties of human metallothionein. Biochemistry 5: 1768-1777.

Rapeseed Association of Canada. 1972. Canadian rapeseed meal in poultry and animal feeding. Publ. 16, Winnipeg, Manitoba, Canada.

Rapeseed Digest. 1971. World production. Rapeseed Assoc. of Canada. Van., B.C. 5, 2:6.

Ritchie, H.D., R.W. Luecke, B.V. Baltzer, E.R. Miller, D.E. Ullrey and J.A. Hoefer. 1963. Copper and zinc interrelationships in the pig. J. Nutr. 79: 117-123.

Saben, H.S. and J.P. Bowland. 1971. Solvent-extracted rapeseed meal as a replacement for soybean meal in diets for swine reproduction. Can. J. Animal Sci. 51: 225-232.

Sacks, A., V.E. Levine, F.C. Hill and R. Hughes. 1943. Copper and iron in human blood. Arch. Intern. Med. 71: 489-501.

Sass-Kortsak, A., M. Chermak, D.W. Geiger and R.J. Slater. 1959. Observation on ceruloplasmin in Wilson disease. J. Clin. Invest. 38: 1672-1682.

Schuld, F.W. and J.P. Bowland. 1968. Dietary rapeseed meal for swine reproduction. Can. J. Animal Sci. 48: 57-64.

Schultze, M.O. 1939. The effect of deficiencies in copper and iron on the cytochrome oxidase of rat tissues. J. Biol. Chem. 129: 729-737.

Schultze, M.O. 1940. Metallic elements and blood formation. Physiol. Rev. 20: 37-67.

Schultze, M.O., C.A. Elvehjem and E.B. Hart. 1936. Studies on the copper and iron content of tissues and organs in nutritional anemia. J. Biol. Chem. 116: 93-106.

Skitsko, P.J. and J.P. Bowland. 1970. Energy and nitrogen digestibility and retention by pigs as influenced by diet, sex, breeding group and replicate. Can. J. Animal Sci. 50: 685-691.

Smith, S.E. and G.H. Ellis. 1947. Copper deficiency in rabbits: Achromotrichia, alopecia and dermatosis. Arch. Bioch. 15: 81-88.

Smith, S.E. and E.J. Larson. 1946. Zinc toxicity in rats and antagonistic effects of copper and liver. J. Biol. Chem. 163: 29-38.

Standish, J.F. and J.P. Bowland. Effects of varying dietary energy, protein and amino acid levels on growth, nutrient digestibility and serum protein levels of early weaned pigs. Can. J. Animal Sci. 47: 77-83.

Starcher, B. 1969. Studies on the mechanism of copper absorption in the chick. J. Nutr. 97: 321-326.

Starcher, B., C.H. Hill and G. Matrone. 1964. Importance of dietary copper in the formation of aortic elastin. J. Nutr. 82: 318-322.

Steel, R.G.D. and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co. Inc., New York. 481 pp.

Sternlieb, I., A.G. Morel, W.D. Tucker, M.M. Green and I.H. Scheinberg. 1960. The incorporation of copper into ceruloplasmin. In vivo studies with copper^{64} and copper^{67}. J. Clin. Invest. 40: 1834-1848.

Suttle, N.F. and C.F. Mills. 1966. Studies of the toxicity of copper to pigs. 1. Effects of oral supplements of zinc and iron salts on the development of copper toxicosis. Brit. J. Nutr. 20: 135-148.

Suttle, N.F. and C.F. Mills. 1966. Studies on the toxicity of copper to pigs. 2. Effect of protein source and other dietary components on the response to high and moderate intakes of copper. Brit. J. Nutr. 20: 149-161.

Taylor, M. and S. Thomke. 1964. Effect of high level copper on some meat and backfat factors of pigs. Xth conference of European meat research workers. Rosklide, pp. 10-15.

Teague, H.S. and L.E. Carpenter. 1951. The demonstration of a copper deficiency in young growing pigs. J. Nutr. 43: 389-399.

Thomke, S. and M. Taylor. 1964. The effect of high level copper on some meat and backfat factors of pigs. Xth conference of European meat research workers. Roskilde. p. 10-15.

Tompsett, S.I. (1940). Factors influencing the absorption of iron and copper from the alimentary tract. Biochem. J. 34: 961.

Ullrey, D.E., E.R. Miller, O.A. Thompson, C.L. Zutaut, D.A. Schmidt, H.D. Ritchie, J.A. Hoefer and R.W. Luecke. 1960. Studies of copper utilization by the baby pigs. J. Animal Sci. 19: 1298 (Abst.).

Underwood, E.J. 1971. Copper. Pp. 57-115 in E.J. Underwood. Tace elements in human and animal nutrition. Third edition; Acad. Press Inc., N.Y.

University of Alberta. 1968. Analysis of variance. Dept. Computing Sci., University of Alberta, Edmonton, Alberta.

Van Campen, D.R. and E.A. Mitchell. 1965. Absorption of Cu^{64}, Zn^{65}, Mo^{99} and Fe^{59} from ligated segments of rat gastrointestinal tract. J. Nutr. 86: 120-124.

Van Campen, D.R. 1966. Effects of zinc, cadmium, silver and mercury on the abosrption and distribution of copper-64 in rats. J. Nutr. 88: 125-130.

Van Campen, D.R. and P.U. Scaife. 1967. Zinc interference with copper absorption in rats. J. Nutr. 91: 473-475.

Van Reen, R. 1953. Effects of excessive dietary zinc in the rat and the interrelationships with copper. Arch. Biochem. Biophys. 46: 337-344.

Virtanen, A.I. 1965. A review study on organic sulphur components and other labile substances in plants. Phytochemistry 4: 207-228.

Wagner, G.R., A.J. Clark, V.W. Hays and V.C. Speer. 1963. Effect of protein-energy relationships on the performance and carcass quality of growing swine. J. Animal Sci. 22: 202-208.

Wallace, H.D. 1967. High level copper in swine feeding: A review of research in the United States. Published by International Copper Research Assn., Inc. New York, N.Y.

Wallace, H.D., R.C. Houser and G.E. Combs. 1966. A high level copper preference study with growing pigs. Dept. Animal Sci. Mimeograph Series No. AN67-2. Florida Agric. Expl. Stn. Gainesville, Florida, U.S.A.

Wallace, H.D., R.H. Houser, A.Z. Palmer, J.W. Carpenter and G.E. Combs. 1966. Response of pigs to high level copper supplementation when

fed in concrete confinement or in small dirt lots. Dept. Animal Sci., Mimeograph Series No. AN67-1. Florida Agric. Expl. Stn., Gainesville, Florida, U.S.A.

Wallace, H.D., R.H. Houser, A.Z. Palmer, J.W. Carpenter, B.R. Cannon and G.E. Combs. 1968. High level copper for pigs fed either soybean meal or fishmeal as the principal protein source. Dept. Animal Sci., Mimeograph Series No. AN69-4. Florida Agric. Expl. Stn., Gainesville, Florida, U.S.A.

Wallace, H.D., J.T. McCall, B. Bass and G.E. Combs. 1960. High level copper for growing-finishing swine. J. Animal Sci. 19: 1153-1163.

Whanger, P.D. and P.H. Weswig. 1970. Effect of some copper antagonists on induction of ceruloplasmin in the rat. J. Nutr. 100: 341-348.

Whanger, P.D. and P.H. Weswig. 1971. Effect of supplementary zinc on the intracellular distribution of hepatic copper in rats. J. Nutr. 101: 1093-1098.

Wynne, K.N. and G.L. McClymont. 1955. Copper-molybdenum-sulphate interaction in induction of hypocuprosis. Nature 175: 471-472.

Young, L.G., G.C. Ashton, R.P. Forshaw and R.H. Ingram. 1968. Relationship of dietary protein levels to performance or carcass merit of market swine. Can. J. Animal Sci. 48: 71-81.

Young, L.G., R.G. Brown, G.C. Ashton and G.C. Smith. 1970. Effect of copper on the utilization of raw soybeans by market pigs. Can. J. Animal Sci. 50: 717-726.

UNIVERSITY OF ALBERTA
LIBRARY

Special Collections

Regulations Regarding Xeroxing of Theses and Dissertations

Typescript copies of theses and dissertations for Master's and Doctor's degrees deposited in the University of Alberta Library, as the official copy of the Faculty of Graduate Studies, may be consulted in the Special Collections Reading Room.

A second copy is generally on deposit in the Department under whose supervision the work was done. Some Departments are willing to loan their copy.

These theses and dissertations are to be used only with due regard to the rights of the author. Written permission of the author must be obtained through the University of Alberta Library when extended passages are copied. Therefore, xeroxing by users is limited to a maximum of twenty pages, and user's are asked to record and sign for the pages xeroxed.

Date Oct 30 Signature Kenneth Gallant Pages copied 6

March 1974